5-1992

The Effects of Feeding a High Level of Rumen Protected Fat with Rumen Undegradable Protein With or Without Niacin on Rumen Fermentation Characteristics, Apparent Nutrient Digestibility, and Milk Production in the Early to Mid Lactation Holstein Cow

Carlos Eduardo Batallas
Utah State University

THE EFFECTS OF FEEDING A HIGH LEVEL OF RUMEN PROTECTED FAT WITH RUMEN UNDEGRADABLE PROTEIN WITH OR WITHOUT NIACIN ON RUMEN FERMENTATION CHARACTERISTICS, APPARENT NUTRIENT DIGESTIBILITY, AND MILK PRODUCTION IN THE EARLY TO MID LACTATION HOLSTEIN COW

by

Carlos Eduardo Batallas

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Dairy Science

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

1992

ACKNOWLEDGEMENTS

Thanks to my parents, Ruth and Eduardo, my wife Sandra, and family who trusted and supported me through the length of my degree. Thanks to Mario and Daeyoon for their unconditional help; to Barb and Paul without whom this learning experience would have been incomplete; to Ron Boman for his academic help and guidance; to Don Sisson for help statistically. Thanks also to my friends, Margaret, Tim, Jane, Billy, Joe, Nell, Joann, Bob, Miles, and Kay, who made my life happier and more bearable in Logan.

My most sincere thanks to Mike J. Arambel who entirely supported me economically and academically to perform the studies presented in this thesis. Without Mike the results obtained from this research would have not been possible.

Gracias a mis padres, Eduardo y Ruth; y a mi familia por creer en mi capacidad. Sin su ayuda y paciencia estos estudios y mi carrera jamas hubiesen sido posibles.

Carlos Batallas

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

THE EFFECTS OF FEEDING A HIGH LEVEL OF RUMEN PROTECTED FAT WITH RUMEN UNDEGRADABLE PROTEIN WITH OR WITHOUT NIACIN ON RUMEN FERMENTATION CHARACTERISTICS, APPARENT NUTRIENT DIGESTIBILITY, AND MILK PRODUCTION IN THE EARLY TO MID LACTATION HOLSTEIN COW

by

Carlos Eduardo Batallas, Master of Science

Utah State University, 1992

Major Professor: Dr. Ronald L. Boman
Department: Animal, Dairy and Veterinary Sciences

Forty high producing early to mid lactation Holstein cows were blocked according to stage of lactation and previous two-week milk yield (experiment 1). Eight ruminally and duodenally cannulated nonlactating Holstein cows were used for experiment 2. The objective was to determine the effects of the ration's high fat content (11.53%) when rumen degradable or rumen undegradable protein is fed with or without added niacin. Cows received one of five treatments: 1) basal ration (TMR); 2) basal ration with added rumen undegradable fat (RUF) (1.6 kg); soybean meal (SBM) (1.73 kg), and niacin (12 g); 3) same as treatment 2, without niacin; 4) same as treatment 2 but replacing the

soybean meal with undegradable protein (UIP) (1.9 kg); and 5) same as treatment 4, without niacin. Rations and water were offered ad-libitum for 10 weeks. Intake for experiment 2 was limited to 18 kg TMR, 0.76 kg RUF, 0.88 kg SBM or UIP, and 6 g niacin. All cows in experiment 2 received the five treatments by the end of five collection periods separated by 21 d adaption. RUF increased dry matter intake (22.95 vs. 23.72 kg/d) and mean body weight (607 vs. 637 kg), but decreased milk protein, lactose and SNF (proportion and yield); and 4% FCM without affecting daily milk yield. RUF, SBM, and N did not affect milk production. Milk protein percentage, protein yield, lactose percentage, SNF percentage, and yield were decreased by UIP supplementation. Niacin had a negative effect on milk fat percentage and yield, and milk protein percentage when fed with RUF and UIP. Supplements did not affect milk protein components. RUF increased plasma glucose (56.8 vs. 63.5). RUF addition increased AD and ND digestibility while decreased fatty acid digestibility. UIP improved ND digestibility in the lactation trial. For experiment 2, UIP increased rumen propionate percentage, thus reducing acetate to propionate ratio. Niacin increased total VFA production (128.6 vs. 114.3 umol/ml). RUF, UIP, and niacin increased total bacterial population. RUF reduced cellulolytic bacteria in rumen fluid. Nutrient rate of passage and digestibility

were unaffected by treatments except for dry matter and RUF digestibility that were reduced by RUF supplementation.

(116 pages)

INTRODUCTION

Dairymen and researchers realize that today's high-producing dairy cow nutritional requirements are difficult to maintain during early lactation when dry matter intake is limited. A high quality ration is necessary to maintain cow production performance and health. Rations formulated to fulfill those requirements must hold a delicate balance of high energy, protein, and other nutrients that may be inadequately provided by microbial synthesis. A potentially detrimental low roughage to concentrate ratio must be avoided to insure proper rumen fermentation, cellulose digestion, milk production and composition, and animal health.

Various studies document the use of supplemental dietary fats to increase the ration's energetic density. They are used to replace a fraction of the grain, thus improving the roughage to concentrate ratio (129). The result usually involves increased fat percent and milk production during early to mid lactation as long as the amount of fat does not exceed 5% of the ration dry matter. Amounts of rumen-available fat above that limit have been shown to be detrimental to rumen fermentation and cellulolytic bacteria, the main acetate producers (35). Diminished acetic acid levels available to rumen wall absorption result in reduced acetate plasma levels reaching the

mammary gland where a large fraction of milk fat must be synthesized from acetate; therefore, reduced milk fat production is observed. Rumen protected fats are recommended since their rumen unavailability reduces their detrimental effects on rumen bacteria, and are more efficient in delivery of fatty acids postruminally. Improved fatty acid supply for duodenal absorption increases plasma fatty acid uptake and transport to the mammary gland. Here long-chain fatty acids are directly transferred to the milk fat fraction. By replacing fatty acids that otherwise would be synthesized at the expense of glucose, rumen protected fats have a sparing effect on plasma glucose, improving its availability for other metabolic mechanisms. Rumen undegradable fat amounts to be supplemented must be restricted to prevent the milk protein depression observed in some studies, particularly now that milk processing plants have begun to stress milk protein content as a base for their pricing procedures (174).

Attempting to maintain milk protein concentration and high milk production, researchers have studied the amount of amino acids provided by microbial protein. Confirmation that microbial synthesis may not produce enough amino acids to maintain high production levels has motivated researchers to study the benefits of feeding undegradable intake protein (UIP). Studies show that postruminal amino acid delivery can be improved by UIP supplementation (146). Scientific

acceptance of this practice becomes evident in the Nutritional Requirements of Dairy Cattle publication by the National Research Council (NRC) where UIP usage recommendations are already present (around 30% of the protein to be fed as UIP) (117).

In addition, other nutrients may have a positive effect on milk protein levels. Researchers have evaluated the effects of niacin supplementation, formerly expected to be sufficiently provided by microbial synthesis. Data from different studies indicate that niacin feeding may have beneficial effects improving performance, body weight gain, nitrogen retention, feed efficiency, and milk production. Furthermore, these results suggest that microbial synthesis does not produce sufficient niacin for animals stressed by high temperatures, high production, or other conditions. In different studies, niacin showed to be helpful to prevent or alleviate early lactation ketosis, and milk protein depression caused by high fat feeding. Niacin may play an important role in energy metabolism, which in turn may be the prime factor to sustain milk protein percent and milk production.

OBJECTIVES

1. To determine the effects of a high fat ration with or without rumen undegradable intake protein with or without niacin on rumen fermentation characteristics, milk production, and nutrient digestibility.

2. To determine the effects of niacin supplementation on milk protein yield, composition, and plasma metabolites.

LITERATURE REVIEW

Current proficient milk production practices require adequate energy delivery to the cow metabolic processes. To achieve efficient energy transfer to milk, fatty acids have been chosen as suitable nutritional supplements due to their high energy concentration. A lactating dairy cow can metabolize up to 32% of its metabolizable energy intake in the form of fatty acids (7). Due to their high energy density, they are utilized to replace a fraction of the grain allowing room to improve the ration's forage to concentrate ratio.

Different types of lipids are available for consumption by dairy cattle. They can be derived from the forage's lipid fraction, grain concentrates, oilseeds, and vegetable and animal lipid byproducts from frying and rendering industries. Industrially processed fat supplements designed to improve handling, storage, and rumen fermentation properties are available.

Fat Supplements

Forages are standard ration ingredients that may contain up to 4% ether extract where only 50% may be actual fatty acids (129). The remainder is comprised of: 1) undigestible cuticular waxes; 2) chlorophyll (a pigment); 3) steroids, which are considered energetically useless (121);

and, 4) nonsaponifiable material such as galactose and glycerol which are fermented in the rumen after hydrolysis. Galactolipide make up 70 to 80% of the crude fat in alfalfa and corn plants (67), with the remaining portion comprised of triacylglycerol (triglyceride). Triglyceride importance becomes evident when phosphate moiety such as phosphorylcholine replaces one of its fatty acids, thus resulting in a phospholipid molecule, an essential part of all living membranes.

Grain ether extract is energetically superior in quality to forage ether extract because it contains about 80% fatty acids with only 20% remaining as non-fatty acid components (67). Polyunsaturated fatty acids are more commonly expected in plant synthesized lipids and are the main components of vegetable oils.

Even though they are also grains, oilseeds are often classified separately when referring to ruminant nutrition. Oilseeds contain up to 20% ether extract and can be used in different ways when fed to improve the energy density of dairy rations. The oilseed's lipid fraction rumen degradability can be modified by processing and feeding management to benefit nutritional practices. Soybeans, cottonseed, sunflower, safflower, and canola seeds are the most investigated oilseeds currently available. Each one contains its own unique fatty acid composition, such as canola seeds. Canola seeds contain higher proportions of

very long chain polyunsaturated fatty acids rarely present in other vegetable oils in significant quantities. Polyunsaturated fatty acids behave singularly during rumen fermentation, gastrointestinal digestion, and milk fat synthesis; special considerations must be made when used in lactating dairy cattle rations. Whole cottonseeds release fatty acids slowly into the rumen (77). Slow release allows linoleic acid (18:2) (about 50% of cottonseed fatty acids) to be either partially or completely biohydrogenated in the rumen, or to bypass rumen fermentation without affecting microbial fermentation. Cottonseed is also a significant source of fiber proven to be important in maintaining fat and milk production (112). Whole raw soybeans have been reported to increase milk production in some studies (127, 133); however, other trials (81) reported a milk fat concentration depression when they were fed with low forage rations, and no change when forage intake was moderate. Raw and toasted soybeans have shown to generate similar production and digestibility responses (137). Raw soybeans were less palatable, and feeding them in large ration proportions led to a milk protein concentration and protein digestibility decrease, probably due to their trypsin inhibitory properties (127). These problems can be avoided by feeding raw soybeans at less than 10% of the total ration DM. Other less uniform and more difficult to handle fat supplements are byproducts from frying and rendering

industries. Different mixtures of vegetable oils, lard, poultry, and animal fat (tallow) are available depending on the geographic area. Several investigative trials have explored their properties, advantages and disadvantages in ruminant rations. The final fatty acid composition of the fat mixtures and their rumen availability have been the ruling factor determining their performance. Palmitic (16:0), oleic (18:1), and stearic acid (18:0) come from animal fat. They are the main components of ruminant adipose tissue and can be synthesized and catabolized by metabolic processes to provide fatty acids for milk fat production during early lactation and body energy storage. Stearate is unique to animal fat and is solid or hard at rumen temperature. Fatty acids with more than one unsaturated bond come from vegetable oils.

Some research has dealt with feeding fish lipids in ruminant rations to modify milk fat composition. Probably, the most important properties of fish oil rely on their fatty acid profile because it contains very long polyunsaturated fatty acids not present in common forages or grains used to nurture dairy cattle. Some of these fatty acids, mainly the omega-3 type, have been associated with beneficial effects on cholesterol, heart disease, thrombosis, child brain development, etc., for human nutrition (69). Very long chain polyunsaturated fatty acids were successfully transferred to the milk fat.

The latest and currently most flourishing group of lipid supplements in the market are the "rumen protected fats." Their main characteristic is rumen undegradability, proven to be beneficial to rumen fermentation by sparing cellulolytic bacteria function and fiber digestion. Calcium salts of fatty acids, prilled fats, and polymer encapsulated lipids are the principal available products. Calcium salts are rumen protected by the pH sensitive bond formed between the metal cation, calcium, and the fatty acid. The bond is stable at normal rumen pH (6.5). However, about 50% of the unsaturated fatty acids are biohydrogenated and saturated in the rumen. Polymer encapsulated lipids come in a small pellet shape surrounded by a pH sensitive polymeric capsula which becomes unstable below pH 5.5. They can successfully deliver fatty acids postruminally and their nutritional performance depends on their rumen biohydrogenation, fatty acid profile, and palatability. Rumen protected lipid supplements contain a wide fatty acid variety. This should be considered when milk fat composition is of importance.

Fat Supplements in the Rumen

Many different strictly anaerobic and some facultative aerobic microorganisms along with protozoa which make up the rumen ecosystem cannot utilize fat as an energy source (67). Fatty acids in conventional diets are mostly esterified and usually rapidly hydrolyzed by rumen lipolytic bacteria (72). They can be taken up by microbes and protozoa (51),

especially those adhered to feed particles (12, 98). Unsaturated fatty acids are biohydrogenated by rumen microbes. They saturate 60 to 90% of the unsaturated bonds forming fully saturated or monounsaturated fatty acids with *trans* configuration (106). *Trans* fatty acids can inhibit milk fat synthesis in the mammary gland. Studies where cows were fed fat supplemented rations showed that rumen microbes can also synthesize 100 to 200 g fatty acids per day (36, 164), but lipids directly incorporated from the diet usually exceed *de novo* synthesis (38). Amount of fatty acid synthesis in the rumen depends on quality and quantity of fatty acids provided by the diet. Significant synthesis has been observed in high or low hay diets (165), whereas supplemented cod liver oil inhibited lipid bacterial synthesis (163). Fat supplementation in the form of oilseeds or dietary fat inhibits *de novo* fatty acid synthesis by bacteria (38) after they have taken up readily available lipids from the rumen environment (52).

Reduced amounts of lipolytic and biohydrogenating bacteria result from high grain-low roughage diets; therefore, the amount of unsaturated fatty acids bypassing the rumen increases (96). Unsaturated fatty acids are more toxic to rumen bacteria than saturated (62, 76). The higher the degree of unsaturation, the more inhibitory the effect of the lipid on microbial growth and rumen fiber

fermentation (85). Intake reduction is a typical sign of decreased fermentation.

Four possible ways by which fatty acids inhibit bacterial growth have been hypothesized: 1) by physical coating of fiber particles thus preventing microbial digestion; therefore, reducing nutrient substrate for growth and reproduction; 2) by reducing fiber digesting rumen bacteria populations; 3) by inhibiting microbial activity due to the surface active effects of fatty acids on cell membranes; and, 4) by reducing cation availability due to the formation of insoluble soaps with long chain fatty acids. The last effect could be indirectly affecting rumen pH by reducing available cations in the rumen (41). Although rumen pH may reduce fiber digestibility, evidence for added fatty acids decreasing pH or cation availability is lacking (15). Bacterial fatty acid uptake is decreased by increased pH in buffered systems (62, 63). This may occur because increasing pH increases ionization, hydro-philicity and, solubility. Bacterial uptake of fatty acids is increased by increasing hydrophobicity (14).

Independent studies show that free fatty acids inhibit rumen bacteria in pure culture (76) and bind to microbial cells (107, 119). Dietary fiber additions reduced binding (68) and inhibition in pure cultures. Bacterial numbers may increase when fat is fed, usually with a protozoal population decrease (35). Protozoa seem to be more easily

depleted by fatty acids, allowing bacteria to move into the empty ecological niche. Adding metal cations, mainly calcium (52), and other alkaline earth metals (Ba, Mg, etc.) appears to help remove fatty acids from microbial cell surface thus effectively reversing fatty acid bactericidal effects (62, 63). However, organic matter digestibility of extracted malt distillers grains with added fatty acids was improved only with Ca (46).

Successive increases of tallow decreased fiber digestibility and rumen ammonia (NH_3) concentrations, suggesting that fiber digestibility was mediated by low NH_3 (89). In other studies, fiber digestibility was not depressed by feeding fat in the diet; overall, fiber digestibility was low but no significant depression was observed (128).

Fatty acids favor propionic acid production at the expense of acetic acid and this may reduce milk fat production (50). Increased amounts of plasma fatty acids reaching the mammary gland may alleviate the reduction. In contrast, fats can be beneficial to rumen fermentation because some bacteria require certain dietary fatty acids for growth (12). Also, fats seem to diminish protein digestibility in the rumen thus improving the amount of dietary amino acids reaching postruminal absorption.

Since rumen microbes do not store triglycerides, the predominant cellular fatty acids are membrane phospholipids

and unesterified fatty acids (171). Exogenous lipid incorporation could spare ATP, which is required for *de novo* fatty acid synthesis. The spared ATP would be available substrate to favor microbial growth and metabolism.

Fat Supplements in the Intestine

The amount of fat entering the small intestine is variable and directly dependent on the ration components consumed. When low fat diets are fed, the amount of lipid reaching the lower gut exceeds dietary supply as a result of microbial synthesis and biliary secretions (114). Fatty acids derived from either microbial or dietary sources reach the intestine 70 to 80% unesterified forming insoluble complexes with particulate matter (70, 151). They must pass through an acid environment (pH 2-3) in the abomasum and the first half of the small intestine where they may become protonated, transferred to solid phase surface, and then rendered accessible for solubilization (151, 154). Decreased pH is mediated by low bicarbonate activity and pancreatic secretions. Approximately in the mid to upper jejunum (2.5 m behind the bile duct in sheep), pH still remains below 5.0 (99). Although this low pH decreases solubility of fatty and bile acids, it favors mineral salts of fatty acid digestion (60, 175) either fed or formed in the rumen. Chemical detachment allows higher absorption of both fatty acid and calcium than would be possible at neutral or alkaline pH. Rumen encapsulated fat supplements

become available for digestion after mild or strong acidification (178).

Free fatty acids, microbial and bile acid, and lysolecithin phospholipid mix with bile salts and pancreatic juices promoting the formation of a colloidal solution of micelles. Bile lipids are 90% phospholipid and the residue is cholesterol, triglycerides, and fatty acids; usually bile acid concentration is twice as much as that of lipids (29). Bile acids are definitely required for fatty acid intestinal absorption in ruminants (74, 75). Their detergent action disperses micelles in the intestinal lumen facilitating lipolysis and mucosa absorption (104, 154). Taurine conjugates predominate in ruminant bile acids. They work at both acid and alkaline pH's (121, 126). Pancreatic lipase is most active at pH 7-8 and requires the presence of phospholipid for lipolysis. Duodenal fluid in ruminants remains at low pH until it reaches the jejunum thus partially delaying fatty acid absorption; however fatty acid absorption takes place before reaching the ileum (121). Pancreatic triglyceride lipase is less active in ruminants than in nonruminants but phospholipase is secreted in excess to alter phospholipid into lysophospholipids which join bile acids and fatty acids to reform micelles and facilitate lipase action. Fatty acid digestibility varies between 83 and 92% (156) in ruminant rations with standard amounts of fat (less than 2% of the dry matter added dietary fat)

(118). The extent to which fatty acids are digested depends on their physical and chemical structure, intestinal dispersability, and amount. Stearic acid usually constitutes the highest fatty acid proportion present in the intestinal lumen due to rumen hydrogenation. Palmitic and monounsaturated acids may be somewhat more digestible than stearate. Flakes of stearic and palmitic acid showed to be only 47% digestible (85) due to lower ruminal and intestinal solubilization. Tallow digestibility is lower do to its high melting point (108). Excessive amounts of fatty acids reaching the small intestine can reduce their digestibility as seen in trials where increasing fat amounts were tested. A ration containing 5.1% crude fat resulted in 81% fat digestibility which decreased to 56% when dietary crude fat percent increased to 10.7% (128). Supplemental fatty acids decrease calcium absorption by 25 to 40% and magnesium about 15% (156). However, extra calcium and magnesium supplementation increased fatty acid absorption in mice and ruminants (17).

Postabsorptive Fat Metabolism

Fatty acids in the form of triglycerides and phospholipids are packed into apoproteins to be transferred from the intestinal lumen into the intracellular space of mucosa cells. Here cholesterol synthesis takes place (114). Lipoproteins, then, are secreted into the lymph (157, 167), which flows to the blood carrying a proportionally balanced

lipid load (121). This insures dietary fat distribution to all tissues proportionally controlled by blood flow (100). Fat absorbed into the portal vein is directed to the liver where triglyceride uptake is limited by the absence of the appropriate enzymes; however, excessive fatty acid circulation tends to accumulate in the liver causing problems (64).

Amount of dietary fat influences lymph and blood lipid composition values (68). Measured esterified fatty acid lymph flow is 200 to 400 g/day. Dietary supplementation of 480 g safflower oil caused a significant increase of lymph flow rate and total lipid transport (71). Heavy by-pass fat supplementation increased total lipid concentration in plasma (179) to a degree where a visible chylomicron layer developed.

Lipoproteins release lipids in contact with lipoprotein lipase (48, 114) in the target tissue's capillary endothelium. Some of the fatty acids are transferred into the tissue cell by mass action and other escape absorption moving on to other tissues or the liver. Lipolytic activity by lipoprotein lipase is sensitive to energy balance and hormones. Adipose lipoprotein lipase is stimulated by insulin (48, 109, 170), while mammary lipoprotein lipase is sensitive to prolactin (50, 166). Both reactions are important in directing triglycerides to either body storage (adipose tissue) or the mammary gland after calving. Target

tissue depend on the cow's energy balance. Adipose tissue is the main fatty acid synthesis site besides the mammary gland (169) during positive energy balance. At this time insulin encourages fat accumulation. During the dry period progesterone levels inhibit fat mobilization. Immediately after parturition, prolactin, growth hormone activity, and negative energy balance promote fatty acid transfer to the plasma and, in turn, the mammary gland. During negative energy balance in early to mid lactation, adipose tissue glycerides are hydrolyzed, reorganized, and released. Fatty acid release overcomes replenishing with the successive body mass loss (45, 48). Studies (16, 49) report mobilization of 30 to 50 kg of fat in the first few weeks of lactation to supply enough energy. This supports a milk production increase of 5 to 6 kg/d for 60 to 90 days.

Mammary gland plasma triglyceride uptake increased linearly when plasma triglyceride concentrations were 30 to 50% above normal levels (10 to 30 mg/dl). Uptake decreased above those levels (5). Thirty to 76% of plasma triglyceride transfer to milk.

Mammary Gland Fat Metabolism

Milk fat composition varies according to stage in lactation, lactation period, dietary, and environmental changes. In general, milk fat is composed of 97 to 98% triglycerols (95), with as many as 437 different fatty acids being isolated (86). The remaining 2 to 3% are

phospholipids, sphingolipids, sterols, fat soluble vitamins, and other minor constituents (95). It has been estimated that about 50% of milk fatty acids are synthesized in the mammary gland from acetate and ß-hydroxybutyrate, 40 to 45% are derived from the diet, while the remaining amounts are derived from adipose tissue (130).

Fatty acids synthesized by the mammary gland are subject to limited variability. These include 16 or less carbon chain fatty acids. On the other hand, blood fatty acids that contribute to the milk fat pool are more sensitive to dietary and environmental conditions. Triacylglycerols and the other milk fat constituents must be assembled from the blood fatty acid pool arising from either *de novo* synthesis or circulating lipids.

Components in blood that contribute to milk fat are glucose, triglycerides of chylomicron and very low density lipoproteins (VLDL), free fatty acids, acetate, and ß-hydroxybutyrate (BHB). Among the free fatty acids, palmitic (16:0), stearic (18:0), and oleic (18:1) are the most available to the mammary gland under normal physiological conditions (73). Lipids may come directly from feed, synthesis of ruminal bacteria, or metabolic products from the cow. Grass fatty acids are mostly long chain and polyunsaturated (93), but are biohydrogenated or fermented by rumen bacteria. In the liver, long chain fatty acids are oxidized to BHB (93). Adipose tissue mobilization provides

triglycerides to blood plasma (73). Ruminal ephithelial cells partially oxidize butyrate to BHB. From the non-lipid ration portion, rumen bacteria ferment dietary carbohydrate to volatile fatty acids: acetate, propionate, butyrate, valerate, isobutyrate, isovalerate, and methylbutyrate (93).

Very low density lipoproteins (VLDL) and low density lipoproteins (LDL) are hydrolyzed by blood capillary endothelial cells, which have a lipoprotein lipase bound to their luminal surface (11). The mammary gland then takes up glycerol, monoglycerides, and free fatty acids by lateral diffusion through the capillary wall into the alveolar cell (120). Mammary gland cells preferentially assimilate stearate containing triglycerides from the plasma triglyceride fraction. The VLDL and LDL contain higher proportions of stearate than linoleate in comparison to the high density lipoproteins (HDL). High density lipoprotein triglycerides are taken up by the mammary gland (73). Mammary gland cells are capable of desaturating many fatty acids that have become saturated in the rumen. Primarily, stearic acid is converted to oleic acid by action of the acyl desaturase enzyme located in the microsomes (13).

Unlike nonruminants, ruminants do not utilize glucose as a carbon source in fatty acid synthesis. The glucose pool for the cow's metabolic processes is limited because the ingested carbohydrates are fermented to volatile fatty acids in the rumen. Glucose produced in the liver and

kidneys by gluconeogenesis is used for lactose synthesis, NADPH generation and glyceride-glycerol formation in the mammary gland. Hence, glucose is diverted to cellular processes where it is an essential precursor (13).

Alterations in the rumen, hence plasma, acetate:propionate ratio induce a glucogenic response of adipose tissue (123) that competes with the mammary gland for acetate, and increases uptake of dietary long chain fatty acids thus causing the "low milk fat syndrome" (37).

The main carbon sources for fatty acid synthesis are acetate and BHB (95). The initial four carbons at the methyl end of each synthesized fatty acid are provided equally by BHB and acetate once these two volatile fatty acids have been converted to CoA derivate. In the case of acetate, it is incorporated as acetyl CoA via the malonyl CoA pathway (161). ß-hydroxybutyrate is predominantly used as the primer unit. In lipogenesis, BHB is cleaved to a 2 carbon molecule by ß-hydroxybutyrate dehydrogenase, an enzyme exclusively found in the mitochondria; then it is converted to acetyl CoA. In the same mitochondria, acetyl CoA is metabolized via the tricarboxylic acid cycle to citrate, isocitrate, α-ketoglutarate, and other compounds. Because the mitochondrial membrane is permeable to citrate, isocitrate and α-ketoglutarate, these compounds are used in the cytol to generate NADPH via the NADP-isocitrate dehydrogenase system. This system is highly active in

ruminants. Another source of reducing equivalents is the pentosephosphate cycle (161). In fatty acid synthesis, the formation of malonyl CoA is the committing step. Because fatty acid synthesis requires bicarbonate, in this step it was possible to elucidate fatty acid synthesis. Insulin and prolactin are acetyl CoA carboxylase activators. Insulin is a short term while prolactin is a long term activator (73). Once acetyl-CoA, malonyl CoA, and NADPH are available in the mammary cytoplasm, the fatty acid synthetase system catalyzes the synthesis of C4 to C16 fatty acids (161). The precise mechanism by which the chain length is regulated is unknown.

Triacylglycerols biosynthesis in the mammary gland appears to be mainly through the glycerol-3-phosphate (G-3-P) pathway (13). The fatty acids to be esterified to the glycerol unit must be in CoA-esters. Synthesized short and medium chain fatty acids are released as such; however long chain fatty acids (C16-C18) are in the free fatty acid form. Long chain fatty acids of milk originate from lipoprotein triglyceride (65%) and nonesterified fatty acids (NEFA) (35%). No net uptake of NEFA by mammary gland is observed (3). Acyl CoA-synthetase esterifies free fatty acids to acyl-CoA (73). Palmquist and Mathos (130) concluded that endogenous sources contributed only 10-12% of the long chain fatty acids in milk fat while the rest came directly from the diet. About 50 to 60% of the G-3-P is generated through

the hexose monophosphate pathway in the mammary gland. Another source of G-3-P could be the blood chylomicra and LDL after phosphorylation by lipoprotein lipase and absorption by the endothelial cells. In glucose metabolism, dihydroxyacetone phosphate (DHAP) is generated. It serves a DHAP source (73).

Other milk lipids include glycerol, phospholipids, sphingolipids, and cholesterol. Most of these are associated with the milk fat globular membrane (93). The phospholipid and sphingolipid pool appears to arise from *de novo* synthesis in the mammary gland (73). Cholesterol seems to be partially synthesized *de novo* with a fraction coming directly from blood serum (13).

Storry and coworkers (160) have reported the effects of fish oils on both rumen fermentation and mammary gland lipid uptake. They observed that mammary uptake of long chain fatty acids was reduced by the uptake of 20-22 C polyunsaturates and further observed an effect of these fatty acids on lipoprotein lipase activity in vitro (21).

Additional studies (158) reported that increased plasma fatty acid uptake by the mammary gland reduced *de novo* synthesis of short chain fatty acids. Increased amounts of long chain acyl-CoA in the mammary gland may inhibit acetyl-CoA carboxylase by a feedback mechanism effectively reducing potential milk fat production. The fact that butyrate concentrations increase during milk depression (155) support

this theory because its synthesis is malonyl CoA formation independent (101).

Fat Supplementation Effects on Milk Production and Composition

In the past, nutritionists assumed that ruminant diets provided enough lipids to maintain production. Banks et al. (8) showed that a low fat diet of 81 g/d limited milk production. Subsequent studies by (8, 14) and other researchers have consistently showed that increasing dietary fat can elevate milk production and modify milk fat composition; however, the type of lipids must be considered (8, 38). Clapperton and Steele (30) and Palmquist and Jenkins (129) reviewed data from different fat feeding trials indicating that supplemental fat improves production within the first twelve weeks of lactation, and to a lesser extent during midlactation. The beneficial effects of feeding fat are more evident with high producing early lactation cows (more than 36.5 kg/d). Cows in this trial (56) received supplement to increase their ration's crude fat from 3.5 to 5.8% inducing a 3.2 kg milk increase during the first 60 d in lactation and 1.4 kg for the next 60 d.

Supplemental fat increases energy intake in early lactation cows at a time when dry matter intake is depressed and production is high. The result appears to be a systemic energy balance improvement that: alleviates hot temperature discomfort (113), lowers blood ketones concentration, spares

glucose utilization by transferring fat directly to milk fat (159), and reduces weight lose during the first seven weeks in lactation.

On the other hand, increased dietary long chain fatty acids (from 18 to 20 C chain) inhibit short chain fatty acid *de novo* synthesis in the mammary gland by reducing lipoprotein lipase activity (47). A homeostatic mechanism to maintain milk fluidity seems to take place because the proportion of *cis* oleate (18:1) increases remarkably (9).

Although researchers (15, 132) have not found significant effects of very long chain polyunsaturated fish oil fatty acids (VLCPUFA) (above 20 C chain) on rumen fermentation, they have observed reduced milk fat levels due to altered mammary gland metabolism and plasma fatty acid profile. Storry et al. (160) reported fish oil's effects on both rumen fermentation and mammary lipid uptake. Mammary gland uptake of long chain fatty acids was reduced by the uptake of 20-22 C polyunsaturates, and lipoprotein lipase activity in vitro was inhibited (21).

Rumen protected fat supplements modify milk fat composition (124) by greatly increasing VLDL triglycerides in plasma, which in turn, increase long chain fatty acid transport to milk fat (179). The increased proportion of long chain fatty acids in milk is underestimated by infrared analysis techniques because the instruments are calibrated to calculate readings based on ester bond quantities (43).

Milk fat mass per bond is higher with long chain fatty acids. Franke et al. (59) recommend adequate calibrations to obtain true milk fat estimations when high amounts of rumen protected fat are fed.

Several studies have reported a milk protein depression in high fat diets (9, 44), especially when most of the fat is present in the protected form. Rations high in rumen protected fat reduced plasma insulin concentration by 25% (103). Insulin is necessary for tissue glucose uptake and appears to be involved in amino acid incorporation into milk protein, but the mammary gland does not require insulin for glucose uptake (103, 147). Mammary gland cells require insulin to mediate casein synthesis (131) by amino acid incorporation into milk protein by mRNA activation. Palmquist and Moser (131) suggest that high plasma fatty acids impair amino acid transport to the mammary gland and protein synthesis by inducing insulin resistance. Insulin resistance is the inability of insulin to stimulate tissue glucose utilization (82). This theory is supported by the fact that high fat feeding mainly affects the casein fraction of milk protein (44). Normal milk protein contains 77 to 81% casein of the total milk nitrogen. Even though researchers have reported a decline of milk protein concentration in cows fed high fat rations (9, 44), total casein production may be the same or higher depending on the total milk yield and is usually improved by moderate fat

supplementation (40). Rations high in rumen protected fat have been reported to shorten the lactation period (155), but the mechanism that produced this result has not been explained yet.

Estimates of NADPH generation from glucose and adipose tissue (6) indicate that direct transfer of fatty acids into milk fat should spare glucose in the pentose phosphate pathway. This could explain how tallow feeding lowered blood ketones concentrations in early lactation. Scientists theorize that glucose is not required to generate reducing equivalents for fatty acid synthesis, but could be diverted to other milk synthesis processes such as lactose synthesis. Lactose synthesis requires glucose (91). A high fat ration can reduce milk lactose concentration (18). Even though nutrient component analysis of these rations demonstrate satisfactory NE_l present, they may be deficient in rumen degradable starch. Starch is the main gluconeogenic precursor (176) when fermented into propionic acid in the rumen.

Protein Supplements

Low and moderate milk production can be supported by microbial protein synthesis (bacteria and protozoa), while increased milk production requires the use of dietary protein supplements that improve the amount of amino acids reaching the small intestine (116, 173). Increased

nutritional requirements are expected during the first eight weeks of lactation (92). These protein supplements must be able to partially escape or bypass rumen microbial proteolysis preserving intact a fraction of their amino acid make up, also, they should degrade postruminally to deliver available amino acids for intestinal absorption.

Dietary protein, when rumen is available, suffers extensive proteolysis producing nutrients valuable to rumen microbial metabolism, namely ammonia (NH_3) and carbon skeletons. Maximum microbial protein synthesis depends on the amount of substrate provided by the ration; however, microflora by itself cannot provide sufficient protein to supply the host's requirements (25, 79, 122, 145). Underestimation of protein requirements to efficiently feed rumen microbes results in diminished microbial protein synthesis, thus reduced microbial protein entering the small intestine (180). Protein from forages is usually highly rumen degradable and can be a very important source of protein for rumen microbes. Alfalfa and grass hay crude protein (CP) is more degradable than silage CP (144). Crude protein from untreated oilseeds and oilseed byproducts (soybean meal) are also rapidly degraded in the rumen.

Rumen undegradability of feed supplements is usually achieved by chemical resistance to microbial attack at rumen pH. Resistance can be induced by three types of treatments: 1) heat (2, 111); 2) formaldehyde spraying (31, 34, 81,

105); and 3) polymeric encapsulation (178). Success of heat and chemical treatment depends upon type of application, concentration, time, and temperature. Misapplication may reduce not only rumen but also overall gastro intestinal (GI) digestibility of the product. The rumen undegradability of polymer capsules depends more on rumen pH and is the most current procedure to deliver individual amino acids postruminally.

Research trials over the last years have established the importance of rumen undegradable proteins for dairy cattle nutrition (10, 26, 24, 58, 142). In the past, protein requirements were described on a CP basis without additional considerations to separate rumen from overall animal performance. The National Research Council (NRC) (116) protein system has been developed based on the recognition that rumen and whole animal nutritional requirements must be considered independently to achieve maximum production and performance with modern dairy cattle. The NRC model considers the degradable intake protein (DIP) and the undegradable intake protein (UIP) fractions of feed. Degradable intake protein is the protein portion available for rumen capture, while UIP is the portion estimated to escape rumen degradation, which includes the total tract undegradable protein in the feed. Nutrient digestibility is affected by rate of passage usually modified by increased dry matter intake (53, 58). Current research trials try to

identify the amino acid requirements for producing dairy cattle. This knowledge will minimize dietary protein wastage and optimize productivity. The same essential amino acids are required by nonruminants and ruminants at the tissue metabolism level (19, 42). Tyrosine and cyst(e)ine have been identified to be essential for milk production (32) but are rarely limiting in lactating rations. Methionine and lysine are the two most limiting amino acids in standard lactating rations (139), but little is known about the ideal combination of amino acids supplied to the small intestine at the absorption site. This may be one of the reasons why various rumen undegradable protein supplements improve milk production (143) and performance (92) while others do not (53, 58). Amino acids are important for different metabolic functions. From the total amino acid pool, 5 to 7% may be used for glucose synthesis through amino acid oxidation (102), and an additional 1% may be used to synthesize carnitine, glutathione, melanin, dopamine, nicotinic acid, and other nonprotein nitrogen compounds. The remaining amino acids are primarily used to synthesize proteins for maintenance, growth, pregnancy, and milk production. Milk protein production is directly affected by: 1) amino acid concentrations in the blood, 2) mammary gland blood flow, and 3) carrier systems (mainly RNA) to transport amino acids across cell membranes. Details are discussed by extensive reviews by Clark et al. (32), Mephan

(110), and Waghorn and Baldwin (172). Once amino acids have been transported successfully into the mammary gland cells, they can be used for synthesis of: milk protein, enzymes, structural proteins, non-essential amino acids, polyamines, or CO_2 (110). Some free amino acids can be readily transferred to milk preserving their free state. Milk protein production cannot be directly predicted from blood amino acid uptake by the mammary gland. Mammary gland metabolism confounded the results in trials that tried to identify uptake vs. output relationships between amino acids and milk protein (110).

Results from different studies start to uncover the production potential of nourishing dairy cows with the adequate amino acid amount and profile. Casein postruminal infusions increased milk production by 4 to 8%, and milk protein yield by 10 to 14% (32) when cows were fed a 17% CP ration. Since the average half-life of amino acids in the rumen is 2 h, protected forms of amino acids must be fed. So far, the best alternative has been offered by the use of polymer-coated compounds carrying 80 to 90% amino acids (150). In cows' early-to-mid lactation, the best results were achieved feeding encapsulated methionine and lysine.

Protected amino acids in their free or compound form become more significant every day; Especially since milk pricing systems start to consider milk protein a more important component of milk. Supplemental amino acids may

play an important role in feeding high producing dairy cows, mainly because including fat in their rations is currently a conventional practice (27). Dietary fat often depresses milk protein concentration (125) affecting mainly casein synthesis, and the depression has been controlled successfully by supplementing niacin (54, 77, 78) and increased levels of UIP (23, 28, 55, 57).

Niacin Supplementation

Niacin or vitamin B_3, the common name for nicotinic acid and nicotinamide, is synthesized by rumen microorganisms (136). Amounts synthesized, in the past, were considered satisfactory to maintain body metabolism and milk production in the lactating cow (79). Afterwards research trials proved that niacin supplementation could improve animal performance under specific conditions. Skaar et al. (153) found that net energy intake was significantly depressed in cows that calved in the warm season compared to the cool season, and niacin supplementation could have beneficial effects on energy balance when dry matter intake is depressed. Recent studies (1, 136) showed that niacin rumen synthesis is affected by the amount of niacin provided in the ration. Niacin's most important function is its role in the coenzyme system NAD+NADP, which is involved in more than 40 metabolic reactions. Enzyme activity regulation,

ATP formulation, carbohydrate, lipid, and protein metabolism depend on that system to take place (161).

Niacin effects on rumen metabolism are erratic. Shields et al. (152) reported microbial growth (especially protozoa (77)) and ammonia utilization enhancement by niacin feeding while Abdouli and Schaefer (1) found no effect on three experiments in vitro. Increased microbial growth would improve microbial protein production and overall performance. It has been suggested that niacin in the rumen may serve as a thiaminase I cosubstrate and precipitate cerebrocortical necrosis.

Morrison et al. (115) reported rapid niacin absorption in the small intestine in humans and an increased pyrridine nucleotide concentration in the blood after continuous dosing with niacin.

Niacin has been reported to improve dry matter intake by 0.8 kg/d with rations containing SBM during early lactation (140); however, the increase may be due to an overall improved energy balance and reduced ketosis. Studies showed that 6 to 12 g/d of niacin maintain higher blood glucose and insulin levels (149) providing the energetic substrate to effectively reduce B-hydroxy butyrate (ketone bodies), an indicator of subclinical and clinical ketosis (20); and that favorable blood metabolite concentrations can be effective for 2 to 5 weeks postpartum (83). The best results have been observed by feeding niacin 1 to 2 week

prepartum to overconditioned cows. Niacin minimizes fatty liver incidence (84, 138) in this type of cattle in early lactation. Niacin is antilipolytic in adipose tissue and reduces nonesterified fatty acids (NEFA) concentrations in plasma (84), which, in turn reduces the amount of triglyceride mobilized to plasma and inhibits liver uptake of long chain fatty acids. The liver is ineffective in metabolizing triglyceride accumulations (88, 128). Lipolytic control of adipose tissue may be the reason why niacin feeding regulates body weight loss (80, 148).

Niacin supplementation has been associated with reversing the milk protein depression caused by dietary fat in the form of whole cottonseed (WCS), soybeans (SB) or calcium salts of fatty acids (Ca-FA) (153). No specific reason has been isolated but a compound effect is suspected from higher dry matter intake, microbial protein, plasma insulin and glucose, reduced ketosis, and improved systemic energy balance (78, 94).

MATERIALS AND METHODS

Experiment 1

Experimental design. Forty multiparous early to mid lactation Holstein cows (75 days in milk average) were assigned to one of five treatment groups (eight cows per group) in a randomized block design. Cows were grouped according to lactation number, stage of lactation, and mean milk production calculated from the two weeks prior to the beginning of the trial. Treatment diets contained: Basal total mixed ration (TMR), sodium-alginate encapsulated rumen undegradable fat (RUF), soybean meal (SBM), blood, meat, and bone meal protein pellet as a source of undegradable intake protein (UIP), and niacin (N). Their distribution and amounts fed on the treatment rations are detailed in Table 1. The basal ration was balanced according to NRC requirements for 600 kg cows producing 40 kg milk/d, and individually fed ad-libitum through Calan gates (American Calan, Inc., Northwood, NH) twice a day (0530 and 1800 h). Feeding was performed by using Calan scale feeders (American Calan, Inc., Northwood, NH) and treatments were top dressed by hand during the morning feeding. Ten percent orts were allowed with intake adjustments performed daily. Cows in

TABLE 1. Treatment supplementation (kg).

Supplement	CTL[1]	SBM[2] + N[3]	SBM	UIP[4] + N	UIP
TMR[5]	ad. lib	ad. lib	ad. lib	ad. lib	ad. lib
RUF[6]	-----	1.60	1.60	1.60	1.60
SBM	-----	1.73	1.73	-----	-----
UIP	-----	-----	-----	1.73	1.73
Niacin, g	-----	12.0	-----	12.0	-----

[1] Control
[2] Soybean meal
[3] Niacin
[4] Undegradable intake protein
[5] Total mixed ration
[6] Rumen undegradable fat

all groups received one kg/d of long stem alfalfa hay at 2300 h. Ration components and analysis are detailed in Tables 2 and 3.

During the 10 weeks of the trial, dry matter intake (DMI), and milk production were monitored daily. Feed, supplements, and orts were sampled weekly, frozen, and later composited for laboratory analysis. Feces were sampled from each cow twice daily for three consecutive days during the last week of the trial to be later composited within cow and analyzed. Feed and feces samples were dried at 60° for 72 h, ground through a Wiley mill (Thomas Wiley Laboratories, Suedesboro, N. J.) equipped with a 1 mm screen, and analyzed for dry matter (DM), crude protein (CP) (66), acid detergent fiber (AD) (168), neutral detergent fiber (ND) (97), fatty acid composition (FA) (162), and acid insoluble ash (AIA) (4). Fatty acid analysis was performed by gas-liquid chromatography (GLC) on a HP 1090A Hewlet Packard equipped with a 182 x 0.64 cm glass column with GP 10% SP-2330 on $^{100}/_{200}$ Chromosorb^{R} (Supelco cat.# 1-1851). Detection was performed with a FID detector fueled with hydrogen at 15 - 20 ml x min^{-1}. Temperatures were set at 220° C at the injector and 225° at the detector. Nitrogen (N_2) was used as a carrier gas at 30 ml x min^{-1} and make-up gas-air mixture flow rate was set at 250 ml x min^{-1}. Oven program started at 130° C. Initial temperature was kept for 10 min. Temperature increased to 195° C at 4°/min. Final time (A)

TABLE 2. Total mixed ration (TMR).

Components	Dry matter %
Alfalfa silage	15.95
Alfalfa hay	31.19
Corn silage	5.34
Rolled barley	16.49
32 % protein pellet[1]	7.16
Whole cottonseed	8.45
Corn distillers grains	4.93
Shredded beet pulp	5.64
Ca salts of fatty acids	1.18
Animal fat	0.78
Limestone	0.30
Cane molasses	0.88
Mineral premix[2]	1.69

[1] Comprised of: crude protein, 32 %; crude fat, 15 %; calcium, 2.5 %; phosphorous, 1.0 %; salt, 2.5 %, Vitamin A (USP units/kg), 66,000; vitamin D3 (USP units/kg),16,500; vitamin E (IU/kg), 66.

[2] Calcium, 8%; potassium, 3.26%; magnessium, 2.2%; sodium, 6%; phosphorus, 5.5%; sulfur, 3.2%; copper, 400 ppm; iron, 3730 ppm; manganese, 2000 ppm; and zinc, 2000 ppm.

TABLE 3. Ration nutrient composition (dry matter).

Nutrient	Basal TMR[1]	RUF[2] + SBM[3]	RUF + UIP[4]
NE_l, Mcal/kg[5]	1.74	1.99	1.99
CP, %	18.08	18.83	18.83
UIP, %	30.00	31.00	47.25
ADF, %	21.10	19.06	18.28
NDF, %	31.11	28.00	26.96
Crude fat, %	6.79	11.53	11.53
Ca, %	1.25	1.11	1.43
P, %	0.51	0.48	0.68
Mg, %	0.26	0.25	0.23
K, %	1.29	1.25	1.12
Ca:P, ratio	2.48	2.31	2.11

[1] Total mixed ration

[2] Rumen undegradable fat

[3] Soybean meal

[4] Undegradable intake protein

[5] Estimated

was 1 min, then temperature was ramped to 250° C at 50°/min with a final time (B) of 1 min. Equilibrium time was also 1 min. Fatty acid extraction was performed as described by Sukhija and Palmquist (162). The internal standard was replaced with pentadecanoic acid (C15:0) because eptadecanoic acid (C17:0) interfered with palmitoleic acid (C16:1) detection. We were unable to separate linolenic (C18:3) from arachidic (C20:0) acid. Both acids were detected at the same retention time, thus were considered together for pertinent calculations. Milk samples were collected weekly to be analyzed for fat, protein, lactose, solids-not-fat (SNF) percent, and somatic cell count (SCC) by the Dairy Herd Improvement (DHI) laboratory (DHIA, Logan, UT) using a Multispec M Infrared Analyzer (Wheldrake, York, England). Additional milk samples at weeks 2, 4, and 6 were frozen (-20° C) to be analyzed for total protein (TP), casein protein (CAS), whey protein (WP), and non protein nitrogen (NPN) (4). Feed, orts, milk, plasma samples, and body weights were collected and recorded the same day every week during the trial before the morning feeding. Blood for plasma samples was obtained from the tail vein into Na-EDTA tubes. Plasma was extracted within three hours after sampling, frozen at -20° C and then composited within cow at the end of the trial to be analyzed for glucose, cholesterol, and BHB using corresponding analysis kits (SIGMA Diagnostics, P.O. Box 14508, St. Louis, Mo).

Weekly mean DMI, feed efficiency, milk production, and 4% fat corrected milk (FCM) were matched with their corresponding weekly fat, protein, lactose, and SNF laboratory analysis (DHI) to compare treatment effects on milk production and components. Nutrient apparent total tract digestibility parameters were calculated using nutrient values obtained from the laboratory analysis. Acid insoluble ash (AIA) values were used as an internal marker.

Statistical analysis. Data was analyzed using ANOVA analysis procedures according to Cochran and Cox (33) with the following models:

Model 1:

$$Y_{ijk} = \mu + T_i + \epsilon_{ij} + W_k + ß_{ijk} + WT_{ik} + \delta_{ijk}$$ where:

Y_{ijk} = the dependent variable (milk yield, composition, components yield, body weight, DMI, feed efficiency, casein, whey protein and NPN)

μ = the overall mean

T_i = effect of $_i$th Treatment

ϵ_{ij} = random effect associated with $_j$th Cow within the $_i$th Treatment (error a)

W_k = effect of $_k$th Week

$ß_{ijk}$ = effect associated with error b

WT_{ik} = effect associated with $_i$th Week x $_k$th Treatment

δ_{ijk} = effect associated with error c for a population arranged in a completely

randomized design (CRD) with repeated measures (split-plot) and the experimental treatments in a 2 x 2 factorial design with an additional treatment (control).

Model 2:

$Y_{ij} = \mu + T_i + \epsilon_{ij}$ where:

Y_{ijk} = the dependent variable (Plasma glucose, ß-hydroxybutyrate, cholesterol; DM, CP, AD, ND, and FA digestibility.

μ = the overall mean

T_i = effect of $_i$th Treatment

ϵ_{ij} = random effect associated with $_j$th Cow within the $_i$th Treatment (error term)

for a population arranged in a completely randomized design (CRD) and the experimental treatments in a 2 x 2 factorial design with an additional treatment (control).

Experiment 2

Experimental design. Eight mature non-lactating Holstein cows fitted with rumen and duodenal cannulae were randomly assigned to one of five treatments (Table 4) in a randomized block design. Rations were designed to proportionally replicate the treatments used for the milk production trial (Experiment 1). Cows received all five treatments by the end of the experiment (five collection

TABLE 4. Treatment supplementation (kg).

Supplement	CTL[1]	SBM[2]+ N[3]	SBM	UIP[4]+ N	UIP
TMR[5]	18	18	18	18	18
RUF[6]	-----	0.76	0.76	0.76	0.76
SBM	-----	0.88	0.88	-----	-----
UIP	-----	-----	-----	0.88	0.88
Niacin, g	-----	6	-----	6	-----

1 Control
2 Soybean meal
3 Niacin
4 Undegradable intake protein
5 Total mixed ration
6 Rumen undegradable fat

periods). Basal ration feeding was divided into two daily feedings (0700 and 1800 h) and supplements were top dressed by hand during the morning feeding.

Five collection periods were completed with a minimum of 21 day adaptation interval. At the end of the interval and five days before each sampling period began, cows were ruminally dosed with 150 g chromium mordanted straw (168) (5 to 10 mm particle size) and subsequent fecal grab samples were collected at 0, 12, 24, 36, 48, 60, 72, 84 and 96 h after dosage. Samples were immediately frozen (-20° C) until the end of the trial then they were oven air dried at 60° C for 3 days and individually ground through a 1 mm screen. Dried and ground fecal samples were then divided into two bags, one bag was reground through a Cyclone mill to improve sample homogeneity for chromium analysis. The remaining bag was used to composite fecal samples within cow and collection period. Composited samples were then analyzed for DM, CP, AD, ND, AIA, and FA to calculate total tract nutrient apparent digestibility.

At the beginning of each sampling period, TMR and supplement samples were collected, oven dried, ground, and stored to be analyzed for DM, CP, AD, ND, AIA, and FA. Cows were fed at 0700 h right after 50 g of NaCo-EDTA (dissolved overnight in 250 ml DI H_2O) had been dosed directly into the rumen through the fistula. Rumen and duodenal fluid samples were collected at 0, 2, 4, 6, 8, 10, and 12 h post-feeding.

Rumen sampling was prolonged to include 24, 36, and 48 h samples after Co-EDTA was dosed to calculate liquid dilution rate (135, 141). Rumen fluid samples were obtained from the ventral sac, pH recorded, strained through two layers of cheese cloth, preserved with 6 N HCl (2 ml HCl + 18 ml rumen fluid) and frozen in plastic 25 ml scintillation vials for later analysis of Cobalt (Co) by atomic absorption spectrophotometry (Buck Scientific) (177), ammonia nitrogen (NH_3-N) (66), volatile fatty acids (VFA) (GLC HP 1090A), and free niacin concentration (0, 2, 4, 8, and 12 h only) (4). From the two hour post-feeding sample, two additional subsamples were separated: 1) 10 ml to be preserved with 10 ml 50% formaldehyde solution to count total protozoal (174); and 2) 20 ml unpreserved to count total and cellulolytic viable bacteria populations (97).

Duodenal samples were collected into 350 ml whirlpak bags directly from the re-entrant cannulae. From each sample, approximately 20 ml of fluid was transferred to a plastic scintillation vial and frozen for later analysis of Co and NH3-N concentration. The remaining sample in the bag was freeze dried, ground (1 mm screen Cyclone mill), and analyzed for CP, AD, ND, and FA concentrations.

Statistical analysis. Data was analyzed using ANOVA analysis procedures according to Cochran and Cox (33) with the following models:

Model 1:

$$Y_{ijk} = \mu + C_i + T_j + CT_{ij} + H_k + CH_{ik} + TH_{jk} + \epsilon_{ijk}$$ where:

Y_{ijk} = the dependent variable (rumen pH, NH3-N, volatile fatty acids (VFA), and niacin)

μ = the overall mean

C_i = effect of $_i$th Cow

T_j = effect of $_j$th Treatment

CT_{ij} = random effect associated with $_i$th Cow within the $_j$th Treatment (error a)

H_k = effect of $_k$th Hour

CH_{ik} = random effect associated with $_i$th Cow x $_k$th Hour

TH_{jk} = effect of $_j$th Treatment x $_k$th Hour

ϵ_{ijk} = random effect associated with error c for a population arranged in a randomized block design with repeated measures (split-plot) and the experimental treatments in a 2 x 2 factorial design with an additional treatment (control).

Model 2:

$$Y_{ij} = \mu + C_i + T_j + \epsilon_{ij}$$ where:

Y_{ijk} = the dependent variable (total and cellulolytic bacteria, protozoa, liquid dilution rate, DM rate of passage, duodenal CP. DM, CP, AD, ND, and FA digestibility)

μ = the overall mean

C_i = effect of $_i$th Cow

T_j = effect of $_j$th Treatment

ϵ_{ij} = random effect associated with $_i$th Cow within the $_j$th Treatment (error term)

for a population arranged in a randomized block design and the experimental treatments in a 2 x 2 factorial design with an additional treatment (control).

RESULTS AND DISCUSSION

Experiment 1

Treatment effects on dry matter intake (DMI), milk yield, milk components, body weights, and feed efficiency are presented in Table 5. Soybean meal, UIP, and niacin supplementation did not affect DMI while RUF addition did (22.95 vs 23.72 kg/d). Dietary fat increased DMI by 0.77 kg/d ($P< 0.05$). This result disagrees with other studies (87, 134) where addition of high levels of RUF decreased DMI. The increase might have been induced by the greater palatability of the fat supplement used in this study (sodium-alginate encapsulated tallow), which appeared to be high. Increased intake, however, did not affect daily milk yield as would be expected with standard rations containing a low or moderate fat concentration. Rumen undegradable fat supplementation to 11.53% of the ration DM, diluted the AD and ND proportions in the treatment rations (see Table 3), thus reducing the amount of rumen fermentable nutrients available for bacterial growth. These results are inconsistent with other studies (65) where supplemental fat increased milk production in early to mid lactation. Since the cows were 75 days in milk (DIM) on average at the beginning of the project, the lactation period where supplemental fat appears to be most effective was probably missed (129). Niacin did not affect daily milk yield with

TABLE 5. Treatment effects on milk yield, components, body weight, dry matter intake (DMI), and feed efficiency.

	Treatments						Effects			
Measure	CTL[1]	SBM[2]+N[3]	SBM	UIP[4]+N	UIP	SEM	C[5] vs F[6]	P[7]	N	P x N
Milk yield, kg/d	33.50	33.67	31.91	31.06	32.37	0.856	--	--	--	**
4% FCM, kg/d	30.90	30.89	29.42	27.27	30.54	0.788	*	--	--	**
Milk fat, %	3.51	3.36	3.45	3.21	3.69	0.073	--	--	**	--
Milk fat, kg/d	1.17	1.13	1.11	0.99	1.17	0.034	*	--	**	**
Milk protein, %	3.08	3.01	3.06	2.95	3.00	0.027	**	**	*	--
Milk protein, kg/d	1.03	1.01	0.97	0.91	0.97	0.024	**	**	--	**
Milk lactose, %	4.92	4.83	4.83	4.81	4.66	0.044	**	**	--	--
Milk lactose, kg/d	1.66	1.64	1.55	1.49	1.52	0.044	**	--	--	--
SNF, %	8.70	8.52	8.56	8.41	8.46	0.048	**	**	--	--
SNF, kg/d	2.92	2.87	2.73	2.60	2.74	0.072	**	*	--	*
Body weight, kg	607	639	663	621	626	7.1	**	**	--	--
DMI, kg/d	22.95	23.87	24.05	23.28	23.66	0.327	**	--	--	--
Feed efficiency[8]	1.35	1.29	1.22	1.17	1.29	0.036	**	--	--	**

[1] Control
[2] Soybean meal
[3] Niacin
[4] Undegradable intake protein
[5] Control
[6] Fat
[7] Protein
[8] 4% FCM/DMI
** $P < 0.05$
* $P < 0.1$

either source of protein (SBM or UIP) to show a significant difference among treatments, but a PxN interaction was identified ($P<0.05$) indicating that niacin behaved differently with SBM and UIP. This result was also observed in the 4% FCM analysis where the same interaction was present. Rumen undegradable fat supplementation reduced 4% FCM by 1.37 kg/d ($P<0.1$).

Dietary RUF, SBM, and UIP did not affect milk fat concentration, while niacin decreased fat proportion by 0.39 percentile points ($P<0,05$) when compared to treatments without niacin. Milk fat yield was decreased ($P<0.1$) by RUF. Niacin decreased fat yield with UIP by 0.18 kg/d ($P<0.01$), and a PxN interaction was again present suggesting that niacin response depended on the source of protein when high fat was fed. RUF also decreased milk protein concentration by 0.07% and protein yield by 0.07 kg/d which has been reported in other studies (22, 65, 90). Soybean meal and UIP did not improve milk protein concentration over the control, but SBM fed cows produced higher milk protein concentration and yield over the UIP treatments ($P<0.05$). Niacin decreased milk protein percent by 0.05 percentile points ($P<0.1$), which is in disagreement with other researchers (80, 153) who reported milk protein concentration improvement by niacin supplementation. On the other hand, niacin did not show an effect on protein yield.

Milk protein breakdown (Table 6) did not reveal any effects from RUF, protein sources, or niacin; however, casein values tended to be low and NPN values tended to be high for all treatments when compared to the milk protein components obtained in other studies (61). Results from this study suggest the control ration (6.79% crude fat) had already depressed milk casein and increased NPN as documented in other studies (39, 44); the experimental treatments did not induce any additional response. Rumen undegradable fat also decreased lactose and SNF concentrations by 0.14 and 0.21 percentile points, respectively (Table 5). Undegradable intake protein treatments produced 0.1 percentile points ($P<0.05$) less lactose than SBM, but did not affect lactose yield. Solids-non-fat concentration decreased 0.1 percentile point by UIP when compared to SBM, and in this case, SNF yield was also affected by protein source where SBM treatments generated 0.13 kg/d ($P<0.1$) more SNF than UIP treatments. Rumen undegradable fat supplementation consistently decreased milk components except for milk fat concentration. High fat appeared to dilute other nutrients thus reducing the amount of substrate available for microbial growth and production. Soybean meal supplementation appeared to alleviate the RUF effect by, probably, increasing readily degradable nutrients necessary for rumen fermentation. This effect might be the reason why SBM treatments outperformed UIP treatments.

TABLE 6. Treatment effects on milk protein composition.

	Treatments						Effects			
Measure	CTL[1]	SBM[2]+N[3]	SBM	UIP[4]+N	UIP	SEM	C[5] vs F[6]	P[7]	N	P x N
Casein, %[8]	85.64	85.53	84.41	83.31	84.14	1.33	--	--	--	--
Whey, %[9]	14.36	14.47	15.59	16.69	15.86	1.33	--	--	--	--
NPN, %[10]	16.94	18.03	16.27	17.19	17.75	0.88	--	--	--	--

[1] Control
[2] Soybean meal
[3] Niacin
[4] Undegradable intake protein
[5] Control
[6] Fat
[7] Protein
[8] % of true milk protein
[9] % of true milk protein
[10] % of milk total nitrogen

Niacin effects are somewhat confusing except for the positive impact on milk protein concentration within protein supplements. PxN interactions mainly occurred in the component yield parameters (milk fat, milk protein, SNF), and feed efficiency, where niacin supplementation was beneficial with SBM and detrimental with UIP. It is suspected that feeding 12 g of niacin might have had a detrimental effect on rumen fermentation when UIP and RUF made up a large proportion of the nutrients in the ration. Cows fed RUF had higher mean body weights than the control cows by 30 kg. Soybean meal fed cows were 28 kg heavier than UIP fed cows ($P<0.05$). Niacin did not affect average body weight within UIP fed cows, but reduced it in SBM fed cows (639 vs. 663 kg), likely due to the antilipolytic properties of niacin described by Jaster et al. (84).

Plasma metabolite analysis (Table 7) showed no effect between treatments for on ß-hydroxybutyrate (BHB) and cholesterol concentrations; however, a PxN interaction was evident in plasma BHB levels ($P<0.05$) indicating a different behavior of niacin with SBM than with UIP. Neither source of protein modified BHB or cholesterol, but there was a difference within UIP treatments due to niacin supplementation, where plasma BHB increased 3.4 mg/dl over UIP treatment without niacin ($P<0.05$). RUF increased plasma glucose by 6.6 mg/dl ($P<0.05$) over the control, probably because of a sparing effect on glucose utilization for fatty

TABLE 7. Treatment effects on plasma metabolites (mg/dl).

	Treatments						Effects			
Measure	CTL[1]	SBM[2]+N[3]	SBM	UIP[4]+N	UIP	SEM	C[5] vs F[6]	P[7]	N	P x N
Glucose	56.83	65.66	64.70	62.35	61.12	2.573	**	--	--	--
β-hydroxybutyrate	8.76	8.74	9.58	11.33	7.93	0.878	--	--	--	**
Cholesterol	207.5	195.1	216.6	192.4	199.1	13.62	--	--	--	--

[1] Control
[2] Soybean meal
[3] Niacin
[4] Undegradable intake protein
[5] Control
[6] Fat
[7] Protein
** $P < 0.05$

acid synthesis in the mammary gland as has been reported by other researchers (18, 90).

Total tract apparent nutrient digestibility is reported in Table 8. Dry matter and CP total tract apparent digestibilities did not differ between treatments (P>.05). Rumen undegradable fat increased CP and ND digestibility 5.93% and 5.47% (P<.05) respectively when compared to the control. This most likely occurred due to the dilution effect caused by RUF addition that decreased fiber concentrations in the treatment rations. Undegradable intake protein enhanced ND digestibility by 3.97% (P<0.1) when compared to SBM rations. Niacin did not affect CP, AD or ND digestibility significantly. This may be due to a slower but more sustained breakdown of the undegradable intake protein. Which in turn allowed improved microbial fiber breakdown (85). Rumen undegradable fat supplementation decreased fatty acid apparent digestibility by 6.01% (P<.01).

Experiment 2

Treatment effects on rumen fermentation characteristics are detailed in Table 9. Rumen undegradable fat supplementation did not affect rumen fluid pH, ammonia-nitrogen, or volatile fatty acids (VFA). Cows fed undegradable intake protein had increased propionate concentration by 1.82% (P<0.1) over SBM rations. This also affected the acetate to propionate ratio (A:P) decreasing it 0.43%

TABLE 8. Treatment effects on total tract nutrient apparent digestibility.

	Treatments						Effects			
Measure	CTL[1]	SBM[2]+N[3]	SBM	UIP[4]+N	UIP	SEM	C[5] vs F[6]	P[7]	N	P x N
DM,[8] %	71.35	70.82	73.86	74.47	77.85	2.380	--	--	--	--
CP,[9] %	71.09	74.40	77.23	76.65	79.80	2.323	**	--	--	--
ADF,[10] %	60.19	60.65	65.29	65.78	70.50	3.080	--	--	--	--
NDF,[11] %	69.71	72.19	74.53	76.46	78.21	2.275	**	*	--	--
FA,[12] %	81.57	70.47	75.80	76.61	79.37	3.025	*	--	--	--

[1] Control
[2] Soybean meal
[3] Niacin
[4] Undegradable intake protein
[5] Control
[6] Fat
[7] Protein
[8] Dry matter
[9] Crude protein
[10] Acid detergent fiber
[11] Neutral detergent fiber
[12] Fatty acid

* $P < 0.1$

** $P < 0.05$

TABLE 9. Treatment effects on rumen fermentation characteristics.

	Treatments						Effects			
Measure	CTL[1]	SBM[2]+N[3]	SBM	UIP[4]+N	UIP	SEM	C[5]vs F[6]	P[7]	N	P x N
pH	6.17	6.30	6.26	6.28	6.31	0.009	--	--	--	--
NH3-N, ug/ml	185.42	214.69	220.47	231.04	188.68	17.47	--	--	--	--
V F A[8]										
Total, umol/ml	122.67	133.64	112.54	123.61	116.04	7.00	--	--	*	--
Acetate, %	65.73	66.21	64.61	64.64	63.88	0.788	--	--	--	--
Propionate, %	16.50	16.39	17.60	18.10	19.53	0.968	--	*	--	--
A : P, ratio	4.10	4.21	3.80	3.71	3.45	0.238	--	*	--	--
Butyrate, %	13.32	13.12	13.07	12.58	12.08	0.544	--	--	--	--
Isobutyrate, %	1.11	1.06	1.19	1.20	1.01	0.076	--	--	--	**
Valerate, %	1.60	1.58	1.73	1.72	1.94	0.141	--	--	--	--
Isovalerate, %	1.71	1.63	1.80	1.75	1.55	0.140	--	--	--	--
Niacin, ug/ml	7.71	23.41	8.80	18.45	9.12	2.54	**	--	**	--

[1] Control
[2] Soybean meal
[3] Niacin
[4] Undegradable intake protein
[5] Control
[6] Fat
[7] Protein
[8] Volatile fatty acids
* $P < 0.1$
** $P < 0.05$

($P<0.1$). Rumen fluid levels of niacin increased with niacin supplementation; however, niacin concentration decreased rapidly post-feeding. Figure 1 illustrates niacin concentration and fluid dilution rate together. Niacin concentration in rumen fluid decreased at a faster rate than Co-EDTA (complex used to measure liquid dilution rate), indicating that niacin was highly degradable in the rumen. If niacin effects depend on its concentration in rumen fluid, or on the amount of niacin passing to the lower gut, a rapid degradation rate would diminish niacin's potential effects. Since all treatment rations except the control contained RUF, statistical analysis showed increased niacin in RUF rations, but this effect was influenced by niacin supplementation, not by fat ($P<0.01$).

Dietary RUF, along with supplemental protein, increased total bacteria in the rumen (Table 10), but decreased cellulolytic bacteria populations by 0.13 and 1.69 log points ($P<0.01$), respectively. The increase in total bacterial populations may be due to the supplemental protein. The decrease in cellulolytic bacteria may be due to the toxic affects of RUF. Undegradable intake protein increased total bacteria by 0.18 log points ($P<0.01$) over SBM. This may be due to a more even release of ammonia into the rumen with the less degradable UIP. Where as with the more degradable SBM, rapid protein breakdown may have resulted in periodic ammonia deficiencies. Niacin tended to

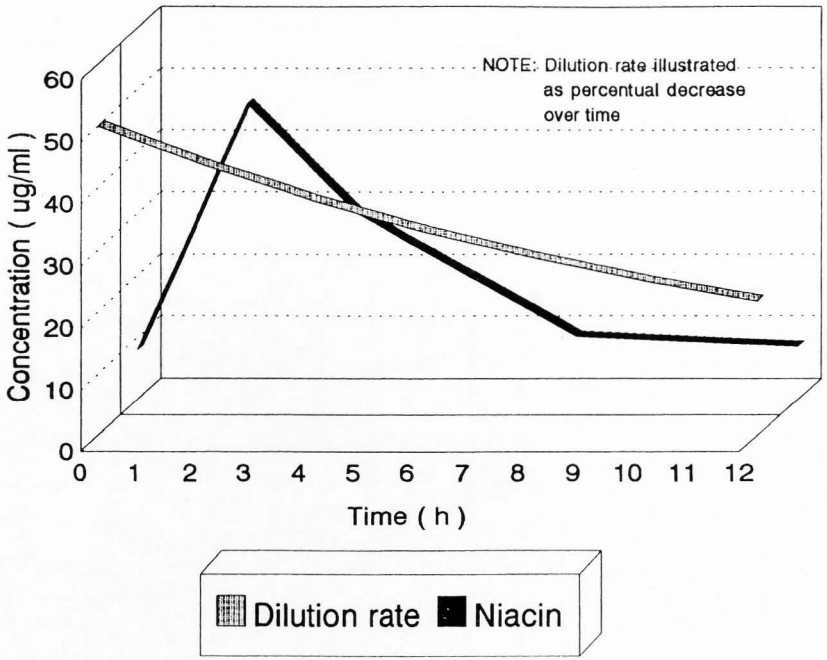

Figure 1. Comparative concentration decrease: Niacin vs. Co-EDTA (dilution rate indicator).

TABLE 10. Treatment effects on rumen microbial populations (log/ml).

	Treatments						Effects			
Measure	CTL[1]	SBM[2]+N[3]	SBM	UIP[4]+N	UIP	SEM	C[5] vs F[6]	P[7]	N	P x N
Bacteria										
Total	10.82	10.84	10.89	10.98	11.10	0.027	**	**	*	--
Cellulolytic	8.73	6.80	7.20	7.313	6.84	0.494	**	--	--	--
Protozoa	5.503	5.388	5.543	5.461	5.471	0.114	--	--	--	--

[1] Control
[2] Soybean meal
[3] Niacin
[4] Undegradable intake protein
[5] Control
[6] Fat
[7] Protein
* $P < 0.1$
** $P < 0.05$

decrease total bacteria populations 0.09 log points ($P<0.1$). These disagree with previous studies (152) where niacin improved rumen fermentation and microbial populations. Protozoa populations were unaffected by treatments. These results disagree with other studies where high levels of dietary fat reduced protozoal numbers and viable cellulolytic bacteria in the rumen (35).

Treatment effects on nutrients delivered to the duodenum are listed in Table 11. Rumen undegradable fat supplementation did not affect NH_3-N levels in fluid samples obtained from the proximal duodenum. Soybean meal containing rations delivered 26.2 ug/ml ($P<0.1$) more NH_3-N than UIP supplemented rations as would be expected when higher proportions of rumen degradable protein (SBM) are fed. Several of the apparent effects of the treatments differed from expectations. One of the reasons for this may be the high levels of fat in the control diet (see Table 2). Crude protein concentration of duodenal solids together with dry matter rates of passage indicate that RUF treatments delivered 1.9% less CP than the control ($P<0.05$). Liquid dilution rate and dry matter rate of passage were not affected by treatments (Table 12).

Total tract apparent nutrient digestibility parameters are described in Table 13. Treatments showed no effect on nutrient digestibility except that RUF decreased dry matter

TABLE 11. Treatment effects on nutrients delivered to the duodenum.

	Treatments						Effects			
Measure	CTL[1]	SBM[2]+N[3]	SBM	UIP[4]+N	UIP	SEM	C[5] vs F[6]	P[7]	N	P x N
NH3-N, ug/ml	89.09	126.40	109.09	94.96	88.21	15.04	--	*	--	--
CP,[8] %	12.51	11.06	10.85	10.07	10.41	0.470	**	--	--	--

[1] Control
[2] Soybean meal
[3] Niacin
[4] Undegradable intake protein
[5] Control
[6] Fat
[7] Protein
[8] % of dry doudenal solid samples
* $P < 0.1$
** $P < 0.05$

TABLE 12. Treatment effects on rate of passage.

	Treatments						Effects			
Measure	CTL[1]	SBM[2]+N[3]	SBM	UIP[4]+N	UIP	SEM	C[5]vs F[6]	P[7]	N	P x N
Liquid dilution rate,[8] %/h	6.37	6.76	6.35	6.69	7.21	0.33	--	--	--	--
Dry matter rate of passage,[9] %/h	1.54	1.54	1.94	2.60	1.87	0.34	--	--	--	--

[1] Control
[2] Soybean meal
[3] Niacin
[4] Undegradable intake protein
[5] Control
[6] Fat
[7] Protein
[8] rumen fluid determination
[9] total tract determination

TABLE 13. Treatment effects on total tract nutrient apparent digestibility.

	Treatments						Effects			
Measure	CTL[1]	SBM[2]+N[3]	SBM	UIP[4]+N	UIP	SEM	C[5] vs F[6]	P[7]	N	P x N
DM,[8] %	70.91	64.30	66.98	63.70	65.74	1.871	**	--	--	--
CP,[9] %	72.63	72.38	74.95	74.30	74.817	1.581	--	--	--	--
ADF,[10] %	57.93	52.45	55.54	51.63	54.50	2.898	--	--	--	--
NDF,[11] %	71.84	68.35	70.83	68.14	70.14	1.933	--	--	--	--
FA,[12] %	76.40	55.99	60.69	54.28	56.85	3.376	**	--	--	--

[1] Control
[2] Soybean meal
[3] Niacin
[4] Undegradable intake protein
[5] Control
[6] Fat
[7] Protein
[8] Dry matter
[9] Crude protein
[10] Acid detergent fiber
[11] Neutral detergent fiber
[12] Fatty acid

** $P < 0.05$

and fatty acid digestibility by 5.73% and 19.44%, respectively ($P<0.05$).

CONCLUSIONS

Experiment 1

In this study an attempt was made isolate the effects of feeding 12 g of niacin with UIP and high levels of RUF. Interactions between niacin and protein sources were evident, mainly indicating that niacin tended to be advantageous with SBM and detrimental with UIP supplementation when high RUF was fed.

Treatment effects were identified though somewhat unclear. The high amount of fat already present in the control ration (see Table 3) may have been a major factor confounding the results. Also, the cows may have been past the period in lactation when RUF, UIP, and niacin are expected to be most effective in improving performance. Nutrient digestibility appeared to be affected by ration composition and rumen fermentation while milk production and composition were not affected by this. Undegradable intake protein, SBM, and niacin interactions and effects on performance need to be better understood when a very high amount of RUF is supplemented. Niacin effects on rumen metabolism must be further studied until actual interactions with other ration components and rumen microbes are understood. Special considerations may be necessary where rumen fermentation may be diminished by high proportions of rumen unavailable-nutrients in the ration.

Experiment 2

In this study an attempt was made to identify the effects of niacin with UIP and high levels of RUF on rumen fermentation characteristics. The lack of clearly identifiable effects by treatments on rumen fermentation parameters suggests the difference was either nonexistent or small enough to be masked by sampling procedures. Overall, this data suggests that the source of UIP was not as rumen undegradable as expected. Niacin supplementation also failed to produce significant results. Niacin's fast concentration decrease in rumen fluid suggests microorganisms rapidly degrade or modify dietary niacin. The effects of niacin on bacterial populations remains unclear. Perhaps a study designed to maintain high niacin levels over time would enhance the effects that could not be isolated in this study.

The RUF (sodium-alginate encapsulated fat) maintained its rumen inertness characteristics. Protozoal populations were spared by RUF supplementation. Studies with other sources of RUF report similar results (65). Microbial populations were affected only to a degree that did not diminish postruminal nutrient delivery or total tract nutrient apparent digestibility.

Additional studies are necessary to isolate the effects of niacin in rumen fermentation when rations contain high proportions of rumen undegradable nutrients, especially

fat and protein. Niacin effects on rumen bacterial populations and fermentation are not well defined and the mechanism by which niacin improved performance in some studies needs to be further investigated.

REFERENCES

1. Abdouli, H., and D. M. Schaefer. 1986. Effect of two dietary niacin concentrations on ruminal fluid free niacin and source of inoculum on in vitro microbial growth, fermentative activity and nicotinamide adenine dinucleotide pool size. J. Anim. Sci. 62:254.

2. Ahrar, M., and D. J. Schingoethe. 1979. Heat-treated soybean meal as a protein supplement for lactating cows. J. Dairy Sci.62:932.

3. Annison, E. F., J. L. Linzell, S. Fazakerley, and B. W. Nichols. 1967. The oxidation and utilization of palmitate, stearate, oleate, and acetate by the mammary gland of the fed goat in relation to their overall metabolism, and the role of plasma phospholipids and neutral lipids in milk-fat synthesis. Biochem. J. 102:637.

4. Association of Official Analytical Chemists. 1980. Official methods of analysis: 13th ed. Assoc. Offic. Anal. Chem. Washington DC.

5. Baldwin, R. I., N. E. Smith, and J. Taylor. 1989. Manipulating metabolic parameters to improve growth rate and milk secretion. Comp. Biochem. Physiol. 96B:411

6. Baldwin, R. L., Y. T. Yang, K. Chist, and G. Grichtng. 1976. Theoretical model of ruminant adipose tissue metabolism in relation to the whole animal. Fed. Proc. 35:2314.

7. Baldwin, R. L., Y. T. Yang, and K. Crist. 1976. Theoretical model of ruminant adipose tissue metabolism in relation to the whole animal. Fed. Proc. 35:2314.

8. Banks, W., J. L. Clapperton, M. E. Ferrie, and A. G. Wilson. 1976. Effect of feeding fat to dairy cows receiving a fat-deficient basal diet. I. Milk yield and composition. J. Dairy Res. 43:213.

9. Banks, W., J. L. Clapperton, and M. E. Ferrie. 1976. Effect of feeding fat to dairy cows receiving a fat-deficient basal diet. II. Fatty acid composition of the milk fat. J. Dairy Res. 43:219.

10. Barney, D. J., D. G. Grieve, G. K. MacLeod., and L. G. Young. 1981. Response of cows to a reduction in dietary

crude protein from 17% to 13% during early lactation. J. Dairy Sci. 68:1352.

11. Barry , J. M., W. Bartley, J. L. Linzell, and D. S. Robinson. 1963. Lipoprotein hydrolyzation in blood cells. Biochem. J. 89:6.

12. Bauchart, D., F. Legay-Carmier, and M. Doreau. 1990. Lipid metabolism of liquid-associated and solid-adherent bacteria in rumen contents of dairy cows offered lipid supplemented diets. Br. J. Nutr. 63:563.

13. Bauman, D. E., and C. L. Davis. 1974. Page 31 in Lactation, B. L. Larson and V. R. Smith, ed. Academic Press, New York, NY.

14. Bean, H. S. 1967. Types and characteristics of disinfectants. J. Appli. Bacteriol. 30:6.

15. Beitz, D. C., and C. L. Davis. 1964. Relationship of certain milk fat depressing diets to changes in the proportions of volatile fatty acids produced in the rumen. J. Dairy Sci. 47:1213.

16. Belyea, R. L., and M. W. Adams. 1990. Energy and nitrogen utilization of high versus low producing dairy cows. J. Dairy. Sci. 73:1023.

17. Bernard, A., M. Fleith, and H. Carlier. 1988. Effect of calcium and magnesium ions on the intestinal absorption of oleic acid in vitro. Reprod. Nutr. Dev. 29:63.

18. Bines, J. A., P. E. Brumby, J. E. Storry, R. J. Fulford, and G. C. Braithwaite. 1978. The effect of protected lipids on nutrient intakes, blood and rumen metabolites and milk secretion in dairy cows during early lactation. J. Agric. Sci. (Camb.) 91:135.

19. Black, A. L., M. Kleiber, and A. H. Smith. 1957. Acetate as a precursor of amino acids of casein in the intact dairy cow. Biochim. Biophys. Acta 23:54.

20. Brent, B. E., and E. E. Bartley. 1984. Thiamin and niacin in the rumen. J. Anim. Sci. 59:813.

21. Brumby, P. E., J. E. Storry, and J. D. Sutton. 1972. Metabolism of cod-liver oil in relation to milk fat secretion. J. Dairy Res. 39:167.

22. Canale, C. J., P. L. Burgess, L. D. Muller, and G. A. Varga. 1990. Calcium salts of fatty acids in diets that differ in neutral detergent fiber: Effect on lactation

performance and nutrient digestibility. J. Dairy Sci. 73:1031.

23. Canale, C. J., L. D. Muller, and H. A. McCahon. 1990. Dietary fat and ruminally protected amino acids for high producing dairy cows. J. Dairy Sci. 73:135.

24. Chalupa, W. 1974. Rumen bypass and protein protection of amino acids. J. Dairy Sci. 58:1198.

25. Chalupa, W. 1976. Degradation of amino acids by the mixed rumen microbial population. J. Anim. Sci. 43:828.

26. Chalupa, W., and J. D. Ferguson. 1988. Recent concepts in protein utilization for ruminants. Page 39 in Southwest Nutr. Conf., Phoenix, Ariz.

27. Chalupa, W., and J. D. Ferguson. 1990. Basic aspects of feeding fat to lactating dairy cattle. In Proc. of the Pacific NW Nutrition Conference, Vancouver, British Columbia.

28. Chow, J. M., E. J. DePeters, and R. L. Baldwin. 1990. Effects of rumen-protected methionine and lysine on casein in milk when diets high in fat or concentrate are fed. J. Dairy Sci. 73:1051.

29. Christie, W. W. 1978. The composition, structure and function of lipids in the tissues of ruminant animals. Prog. Lipid Res. 17:111.

30. Clapperton, J. L., and W. Steele. 1983. Fat supplementation in animal production ruminants. Proc. Nutr. Soc. 42:343.

31. Clark, J. H., C. L. Davis, and E. E. Hatfield. 1974. Effect of formaldehyde treated soybean meal on nutrient use, milk yield, composition and free amino acids in lactating bovine. J. Dairy Sci. 57:1031.

32. Clark, J. H., H. R. Spires, and C. L. Davis. 1978. Uptake and metabolism of nitrogenous components by the lactating mammary gland. Fed. Proc. 37:1233.

33. Cochran, W. G., and G. M. Cox. 1950. Experimental Designs. John Wiley & Sons, Inc., New York, NY.

34. Crooker, B. A., J. H. Clark, and R. D. Shank. 1983. Effects of formaldehyde treated soybean meal on milk yield, milk composition and nutrient digestibility in the dairy cow. J. Dairy Sci. 66:492.

35. Czerkawski, J. W. 1973. Effect of linseed oil fatty acids and linseed oil on rumen fermentation in sheep. J. Agric. Res., Camb. 81:517.

36. Czerkawski, J. W., and J. L. Clapperton. 1984. Fats as energy yielding compounds in the ruminant diet. Page 249 in Fats in Animal Nutrition. J. Wiseman, ed. London, Butterworths.

37. Davis, C. L. and R. E. Brown. 1970. Low-fat milk syndrome. In Physiology of Digestion and Metabolism in the Ruminant. A. T. Phillipson, ed. Oriel Press, Newcastle-upon-Tyne, England.

38. Demeyer, D. L., C. Henderson, and R. A. Prins. 1978. Relative significance of exogenous and *de novo* synthesized fatty acids in the formation of rumen microbial lipids in vitro. Appl. Environ. Microbiol. 35:24.

39. DePeters, E. J., S. J. Taylor, A. A. Franke, and A. Aguirre.1985. Effects of feeding whole cottonseed on composition of milk. J. Dairy Sci. 68:897.

40. DePeters, E. J., S. J. Taylor, C.M. Finley, and T. R. Famula. 1987. Dietary fat and nitrogen composition of milk from lactating cows. J. Dairy Sci. 70:1192.

41. Devendra, C., and D. Lewis. 1974. The interaction between dietary lipids and fiber in the sheep. Anim. Prod. 19:67.

42. Downes, A. M. 1961. On the amino acids essential for the tissues of the sheep. Aust. J. Biol. Sci. 14:254.

43. Dunkley, W. L., A. A. Franke, and L. M. Smith. 1977. Determining fat, protein, and lactose in milk from cows fed formaldehyde protected sunflower-soybean supplement. J. Dairy Sci. 60:45.

44. Dunkley, W. L., N. E. Smith, and A. A. Franke. 1977. Effect of feeding protected tallow on composition of milk and milk fat. J. Dairy Sci. 60:1863.

45. Dunshea, F. R., A. W. Bell, and T. E. Trigg. 1990. Non-esterified fatty acid and glycerol kinetics and fatty acid re-esterification in goats during early lactation. Br. J. Nutr. 64:133.

46. El Hang, G. A., and T. B. Miller. 1972. Evaluation of whisky distillery by-products. J. Sci. Food Agric. 23:247.

47. Emery, R. S. 1978. Feeding for increased milk protein. J. Dairy Sci. 61:825.

48. Emery, R. S. 1979. Deposition, secretion and oxidation of fats in ruminants. J. Anim. Sci. 48:1530.

49. Emery, R. S. 1988. Feed intake and change in body composition of lactating mammals. ISI Atlas of Science: Animal and Plant Sciences 1:51.

50. Emery, R. S. 1988. Milk fat depression and the influence of diet on milk composition. Vet. Clin. North Am. (Food Anim. Pract.) 4:289.

51. Emmanuel, B. 1974. On the origin of rumen protozoan fatty acids. Biochim. Biophs. Acta. 337:404.

52. Emmanuel, B. 1978. The relative contribution of propionate, and long chain even-numbered fatty acids in the production of long chain odd-numbered fatty acids in rumen bacteria. Biochim. Biophs. Acta. 528:239.

53. Erdman, R. A., and J. H. Vandersall. 1983. Effect of rumen protein degradability on milk yield of dairy cows in early lactation. J. Dairy Sci. 66:1873.

54. Erickson, P. S., M. R. Murphy, and J. H. Clark. 1989. Calcium salts of long chain fatty acids and nicotinic acid supplementation of diets fed to Holstein cows in early lactation. J. Dairy Sci. 72(Suppl. 1):483.

55. Ferguson, J. D., C. J. Sniffen, and C. J. Muscato. 1988. Effects of protein degradability and protected fat supplementation on milk yield on dairy cows. J. Dairy Sci. 72(Suppl. 1):415.

56. Ferguson, J. D., D. Sklan, and W. Chalupa. 1990. Effects of hard fats on in vitro and in vivo rumen fermentation, milk production and reproduction in dairy cows. J. Dairy Sci. 73:2864.

57. Ferguson, J. D., J. Torralba, and P. L. Schneider. 1988. Response of lactating cows in commercial dairies to calcium salts of long chain fatty acids. J. Dairy Sci. 71(Suppl. 1):254.

58. Forster, R. J., D. G. Grieve, J. G. Buchanan-Smith, and G. K. MacLeod. 1983. Effect of dietary protein degradability on cows in early lactation. J. Dairy Sci. 66:1653.

59. Franke, A. A., W. L. Dunkley, and N. E. Smith. 1977. Influence of feeding protected tallow on accuracy of analysis by infrared milk analyzer. J. Dairy Sci. 60:1870.

60. Freeman, C. P. 1969. Properties of fatty acids in dispersions of emulsified lipid and bile salts and the significance of these properties in fat absorption in the pig and the sheep. Brit. J. Nutr. 23:249.

61. Fronk, T. J., and L. H. Schultz. 1979. Oral nicotinic acid as a treatment for ketosis. J. Dairy Sci. 62:1804.

62. Gallbraith, H., and T. B. Miller. 1973. Effect of metal cations and pH on the antibacterial activity and uptake of long chain fatty acids. J. Appl. Bacteriol. 36:635.

63. Gallbraith, H., T. B. Miller., A. M. Patton, and J. K. Thompson. 1971. Antibacterial activity of long chain fatty acids and the reversal with calcium, magnesium, ergocalciferol and cholesterol. J. Appl. Bacteriol. 34:803.

64. Gerloff, B. J., T. J. Herdt, and R. S. Emery. 1986. Relationship of hepatic lipolysis to health and performance in dairy cattle. J. Am. Vet. Med. Assoc. 188:845.

65. Grummer, R. R. 1988. Influence of prilled fat and calcium salts of palm oil fatty acids on ruminal fermentation and nutrient digestibility. J. Dairy Sci. 71:117.

66. Hach Company, 1989. Instructional Manual: Digestahl Digestion Apparatus, model 32130-20.

67. Harfoot C. G. 1978. Lipid metabolism in the rumen. Prog. Lipid Res. 17:21.

68. Harfoot, C. G., M. L. Crouchman, R. C. Noble , and J. H. Moore. 1974. Competition between food particles and rumen bacteria in the uptake of long chain fatty acids and triglycerides. J. Appl. Bacteriol. 37:633.

69. Harris, W. S. 1989. Fish oils and plasma lipid and lipoprotein metabolism in humans: A critical review. J. Lipid Res. 30:785.

70. Harrison, F. A., and W. M. F. Leat. 1972. Absorption of palmitic, stearic and oleic acids in the sheep in the presence or absence of bile and/or pancreatic juice. J. Physiol. 225:565.

71. Hartmann, P. E., J. G. Harris, and A. K. Lascelles. 1966. The effects of oil feeding and starvation on the composition and output of lipid in thoracic duct lymph in the lactating dairy cow. Aust. J. Biol Sci. 19:635.

72. Hawke, J. C., and W. R. Silcock. 1970. The in vitro rates of lipolysis and rumen biohydrogenation in rumen contents. Biochim. Biophs. Acta 218:201.

73. Hawke, J. C. and M. W. Taylor. 1983. Page 37 in Developments *in* Dairy Chemistry. P. F. Fox, ed. Applied Sci Publishers, New York, NY.

74. Heath, T. J., and L. N. Hill. 1969. Dietary and endogenous long chain acids in the intestine of sheep, with an appendix on their estimations in feeds, bile and faeces. Australian J. Biol. Sci. 22:1015.

75. Heath, T. J., and B. Morris. 1963. The role of bile and pancreatic juice in the absorption of fat in ewes and lambs. Brit. J. Nutr. 17:465.

76. Henderson, C. 1973. The effect of fatty acids on pure culture of rumen bacteria. J. Agr. Sci. (Camb.) 81:107.

77. Horner, J. L., C. E. Coppock, and J. R. Moya. 1988. Effects of niacin and whole cotton seed on ruminal fermentation, protein degradability, and nutrient digestibility. J. Dairy Sci. 71:1239.

78. Horner, J. L., C. E. Coppock, G. T. Schelling, J. L. LaBore, and D. H. Nave. 1986. Influence of niacin and whole cottonseed on intake, milk yield and composition, and systemic responses of dairy cows. J. Dairy Sci. 69:3087.

79. Hungate, R. E. 1966. The rumen and its microbes. Academic Press, New York, NY.

80. Hutjens, M. F. 1990. Niacin in dairy cattle. In NFIA Nutrition Institute Proceedings, Kansas City, MO.

81. Hutjens, M. F., and L. H. Schultz. 1971. Addition of soybeans or methionine analog to high concentrations for dairy cows. J. Dairy Sci. 54:1637.

82. Ip, C., H. Tepperman, P. Holohan, and J. Teperman. 1976. Insulin binding and insulin response of adipocytes from rats adapted to fat feeding. J. Lipid Res. 17:588.

83. Jaster, E. H. 1988. Nicotinic acid or nicotinamide for lactating dairy cows. Page 3 in Proc. of the Degussa Technical Symposium, Atlanta, GA.

84. Jaster, E. H., D. F. Bell, and T. A. McPherron. 1983. Nicotinic acid and serum metabolite concentrations of lactating dairy cows fed supplemental niacin. J. Dairy Sci. 66:1039.

85. Jenkins, T. C., and B. F. Jenny. 1989. Effect of hydrogenated fat on feed intake, nutrient digestion, and lactation performance of dairy cows. J. Dairy Sci. 72:2316.

86. Jennes, R. 1985. Page 129 in Lactation. B. L. Larson, ed. Iowa State University Press, Ames IA.

87. Kincaid, R. L., J. P. McNamara, J. H. Harrison, and S. S. Waltner. 1991. Effects of cottonseed and calcium salts of long chain fatty acids on performance of lactating dairy cows. J. Dairy Sci. 74(Supp. 1):252.

88. Kleppe, B. B., R. J. Aiello, R. R. Grummer, and L. E. Armentano. 1988. Triglyceride accumulation and very low density lipoprotein secretion by rat and goat hepatocytes in vitro. J. Dairy Sci. 71:1813.

89. Kowalczyk, J., E. R. ϕrskov, J. J. Robinson, and C. S. Stewart. 1977. Effect of fat supplementation on voluntary food intake and rumen metabolism in sheep. Brit. J. Nutr. 37:251.

90. Kronfeld, D. S., Donoghue, J. M. Naylor, K. Johnson, and C. A. Bradley. 1980. Metabolic effects of feeding protected tallow to dairy cows. J. Dairy Sci. 63:545.

91. Kuhn, N. J., D. T. Carrick, and C. J. Wilde. 1980. Lactose synthesis: possibilities of regulation. J. Dairy Sci. 63:328.

92. Kung, L. Jr., and J. T. Huber. 1983. Performance of high producing dairy cows in early lactation fed protein of varying amounts, sources, and degradability. J. dairy Sci. 66:227.

93. Kurtz, F. E. 1974. Page 125 in Fundamentals of Dairy Chemistry. AVI Publ. Co., Inc., Westport, CT.

94. Lanham, J. K., C. E. Coppock, D. L. Wilks, K. N. Brooks, and J. L. Horner. 1988. Effects of whole cottonseed and

(or) niacin on lactating Holstein cows in hot weather. J. Dairy Sci. 71(Suppl. 1):253. (Abstr.)

95. Larson, B. L. 1985. Page 129 in Lactation, B. L. Larson, ed. Iowa State University Press, Ames, IA.

96. Latham, M. J., E. Storry, and M. E. Sharpe. 1972. Effect of low-roughage diets on the microflora and lipid metabolism in the rumen. Appl. Microbiol. 24:871.

97. Leedle, J. A. Z., and R. B. Hespell. 1980. Differential carbohydrate media and anaerobic replica plating techniques in delineating carbohydrate-utilizing subgroups in rumen bacterial populations. Appl. and Environ. Microb. 39:709.

98. Legay-Carmier, F., and D. Bauchart. 1989. Distribution of bacteria in the rumen contents of dairy cows given a diet supplemented with soya-bean oil. Br. J. Nutr. 61:725.

99. Lennox, A. M., and G. A. Garton. 1968. The absorption of long-chain fatty acids from the small intestine of sheep. Brit. J. Nutr. 22:247.

100. Liesman, J., R. S. Emery, and B. Gerloff. 1984. Salt-resistant triglyceride lipase related to post-partum disease. Can J. Anim. Sci. 64(Suppl):248.

101. Lin, C. Y., and S. Kumar. 1972. Pathway for synthesis of fatty acids in mammalian tissues. J. Biol. Chem. 247:604.

102. Lindsay, D. B. 1982. Relationships between amino acid catabolism and protein anabolism in the ruminant. Fed. Proc. 41:2550.

103. Lomax, M. A., G. D. Baird, C. N. Mallison, and H. W. Symonds. 1979. Differences between lactating and non-lactating dairy cows in concentration and secretion rate of insulin. Biochem. J. 180:281.

104. Lough, A. K., and A. Smith. 1976. Influence of the products of phospholipolysis of phosphatidylcholine on micellar solubilization of fatty acids in the presence of bile salts. Brit. J. Nutr. 35:89.

105. Lundquist, R. G., D. E. Otterby, and J. G. Linn. 1986. Influence of formaldehyde-treated soybean meal on milk production. J. Dairy Sci. 69:1337.

106. Mattos, W., and D. L. Palmquist. 1977. Biohydrogenation and availability of linoleic acid in lactation cows. J. Nutr. 107:1755.

107. Maxcy, R. B., and C. W. Dill.1967. Absorption of free fatty acids on cells of certain microorganisms. J. Dairy Sci. 50:472.

108. McLeod, G. K., and J. Buchanan-Smith. 1972. Digestibility of hydrogenated tallow, saturated fatty acids and soybean oil- supplemented diets by sheep. J. Anim. Sci. 35:890.

109. McNamara, J. P., and J. K. Hillers. 1986. Regulation of bovine adipose tissue metabolism during lactation. 1. Lipid synthesis in response to increased milk production and decreased energy intake. J. Dairy. Sci 69:3032.

110. Mephan, T. B. 1982. Amino acid utilization by lactating mammary gland. J. Dairy Sci. 65:287.

111. Mielke, C. D., and J. Schingoethe. 1981. Heat-treated soybeans for lactating cows. J. Dairy Sci. 64:1579.

112. Moody, E. G. 1978. Cottonseed and oil in dairy rations at two roughage levels. Feedstuffs 50(41):20.

113. Moody, E. G., J. P. VanSoest, R. E. McDowell, and G. L. Ford. 1967. Effect of high temperature and dietary fat on performence of lactating cows. J. Dairy Sci. 50:1909.

114. Moore, J. H. and W. W. Christie. 1984. Digestion, absorption and transport of fats in ruminant animals. Page 123 in Fats in Animal Nutrition, J. Wiseman, ed. London, Butterworths.

115. Morrison, M. A., M. S. Reynolds, and A. E Harper. 1963. Effects of increments of tryptophan and niacin on growth and on the concentration on blood and liver pyridine dinucleotides. J. Nutr. 63:441.

116. National Research Council. 1985. Ruminant nitrogen usage. 6th ed. Natl. Acad. Sci., Washington, DC.

117. National Research Council. 1989. Nutrient requirements of dairy cattle. 6th ed. Natl. Acad. Sci., Washington, DC.

118. Ngidi, N. M., S. C. Lorch, and F. L. Fluharty. 1990. Effects of calcium salts of long chain fatty acids on feed lot performance, carcass characteristics of ruminal metabolism of steers. J. Anim. Sci. 68:2555.

119. Nieman, C. 1954. Influence of trace amounts of fatty acids on the growth of microorganisms. Bacteriol. Rev. 18:147.

120. Nilsson-Ehle, P., A. S. Garfunkel, and M. C. Scholtz. 1980. Ann. Rev. Biochem. 49:667.

121. Noble R. C. 1978. Digestion, absorption and transport of lipids in ruminant animals. Prog. Lipid Res. 17:55.

122. ϕrskov. E. R., C. Fraser, and E. L. Corse. 1970. The effect on protein utilization of feeding different protein supplements via the rumen or via the abomasum in young growing sheep. Brit. J. Nutr. 24:803.

123. Palmquist, D. L. 1976. Ruminant adipose tissue metabolism. Fed. Proc. 35:2300.

124. Palmquist, D. L., 1978. A kinetic concept of lipid transport in ruminants. A review. J. Dairy Sci. 59:355.

125. Palmquist, D. L. 1984. Use of fats in diets for lactating cows. Page 357 in Fats in Animal Nutrition, J. Wiseman, ed. Boston, Butterwoths.

126. Palmquist, D. L. 1988. The feeding value of fat. In Feed Science, Orskow ER, ed. Amsterdam, Elsevier.

127. Palmquist, D. L., and H. R. Conrad. 1971. High levels of raw soybeans for dairy cows. J. Anim. Sci. 33:295. (Abstr.).

128. Palmquist, D. L., and H. R. Conrad. 1978. High fat rations for dairy cows. Effects on feed intake, milk and fat production, and plasma metabolites. J. Dairy Sci. 61:890.

129. Palmquist, D. L., and T. C. Jenkins. 1980. Fat in lactating rations: review. J. Dairy Sci. 63:1.

130. Palmquist, D. L., and W. Mattos. 1978. Turnover of lipoproteins and transfer to milk fat of dietary (1-Carbon-14) linoleic acid in lactating cows. J. Dairy Sci. 61:561.

131. Palmquist, D. L., and E. A. Moser. 1981. Dietary fat effects on blood insulin, glucose utilization, and milk protein content of lactating dairy cows. J. Dairy Sci. 64:1664.

132. Pennington, J. A., and C. L. Davis 1975. Effects of intraruminal and intraabomasal additions of cod liver

oil on milk fat production in the cow. J. Dairy Sci. 58:49.

133. Perry, F. G., and G. K. MacLeod. 1968. Effects of feeding raw soybeans on rumen metabolism and milk composition of dairy cows. J. Dairy Sci. 51:1233.

134. Pitcher, P. M., J. D. Ferguson, D. T. Galligan, and W. Chalupa. 1991. Responses of primiparous cows to calcium salts of long chain fatty acids. J. Dairy Sci. 74:(Supp. 1):253.

135. Pond, K. R., J. C. Burns, and D. S. Fisher. 1987. External markers - use and methodology in grazing studies. Proc. Grazing Livestock Nutr. Conference. July 23-24.

136. Porter, J. W. G. 1961. Vitamin synthesis in the rumen. Page 226 in Digestive Physiology and Nutrition of the Ruminant, D. Lewis, ed. Butterwoths, London.

137. Rakes. A. H., D. G. Davenport, and G. R. Marshall. 1972. Feeding value of roasted soybeans for dairy cows. J. Dairy Sci. 55:529.

138. Reid, I. M., and C. J. Roberts. 1983. Subclinical fatty liver in dairy cows. Ir. Vet. J. 37:104.

139. Richard C. R., and E. E. Hatfield. 1978. The limiting amino acid in growing cattle. J. Anim. Sci. 46:740.

140. Riddell, D. L., E. E. Bartley, A. D. Dayton. 1981. Effect of nicotinic acid on microbial protein synthesis in vitro and on dairy cattle growth and milk production. J. Dairy Sci. 64:782.

141. Rittenhouse, L. R., D. E. Johnson, and M. M. Borman. 1982. Comparison of fecal excretion and intake estimates obtained from external markers and total collection. Proc. West. Sect. Am. Soc. Anim. Sci. 33:284.

142. Rock, D., J. Kalter, T. Klopfenstein, and R. Britton. 1979. Slowly degraded protein sources. Neb. Beef Cattle Report. EC. 79:11.

143. Ruegsegger, G. J., and L. H. Schultz. 1985. Response of high producing dairy cows in early lactation to the feeding of heat-treated whole soybeans. J. Dairy Sci. 68:3272.

144. Satter, L. D. 1985. Interrelationships of dietary energy, protein and nonprotein nitrogen concentrations

and performance of lactating cows. Page 142 in Proc. Georgia Nutr. Conf. Univ. Georgia, Athens.

145. Satter, L. D., and R. E. Roffler. 1974. Nitrogen requirement and utilization in dairy cattle. J. Dairy Sci. 58:1219.

146. Schingoethe, D. J., D. P. Casper, C. Yang, D. J. Illg, J. L. Sommerfeldt, and C. R. Mueller. 1988. Lactational response to soybean meal, heated soybean meal, and extruded soybeans with ruminally protected methionine. J. Dairy Sci. 71:173.

147. Schmidt, G. H., 1966. Effect of insulin on yield and composition of milk of dairy cows. J. dairy Sci. 49:381.

148. Schultz, L. H. 1983. Niacin in dairy rations. In Pacific N. W. Nutrition Conference Proceedings, Corvallis, OR.

149. Schwab, C. G. 1983. Supplemental niacin for lactating dairy cows. In Proc. of the 24th Annual New England Dairy Feed Conference, Concord, NH.

150. Schwab, C. G. 1989. Amino acids in dairy cow nutrition. Anim. Nutr. Technical Symposium. California Anim. Nutr. Conf. March 15, 1989.

151. Scott, A. M., and A. K. Lough, 1971. The influence of biliary constituents in an acid medium on the micellar solubilization of unesterified fatty acids of the duodenal digesta of sheep. Brit. J. Nutr. 25:307.

152. Shields, D. R., D. M. Schaefer, and T. W. Perry. 1983. Influence of niacin supplementation and nitrogen source on rumen microbial fermentation. J. Anim. Sci. 57:1576.

153. Skaar, T. C., R. R. Grummer, M. R. Dentine, and R. H. Stauffacher. 1989. Seasonal effects of prepartum and postpartum fat and niacin feeding on lactation performance and lipid metabolism. J. Dairy Sci. 72:2028.

154. Smith, A., and A. K. Lough. 1976. Micellar solubilization of fatty acids in aqueous media containing bile salts and phospholipids. Brit. J. Nutr. 35:77.

155. Smith, N. E., W. L. Dunkley, and A. A. Franke. 1978. Effects of feeding protected tallow to dairy cows in early lactation. J. Dairy Sci. 61:747.

156. Steele, W. 1983. Intestinal absorption of fatty acids and blood lipid composition in sheep. J. Dairy Sci. 66:520.

157. Sterzing, P. R., A. D. McGilliard, and R. S. Allen, 1971. Ultrastructural aspects of lipid absorption by bovine intestinal mucosa. J. Dairy Sci. 54:1436.

158. Storry, J. E., A. J. Hall, and V. W. Johnson. 1973. The effects of increasing amounts of dietary tallow on milk-fat secretion in the cow. J. Dairy Res. 40:293.

159. Storry, J. E., P. E. Brumby, A. J. Hall, and V. W. Johnson. 1974. Responses in rumen fermentation and milk-fat secretion in cows receiving low forage diets supplemented with protected tallow. J. Dairy Res. 41:165.

160. Storry, J. E., P.E. Brumby, A. J. Hall, and B. Tuckley. 1974. Effects of free and protected forms of cod liver oil on milk fat secretion in the dairy cow. J. Dairy Sci. 57:1046.

161. Stryer, L. 1988. Biochemistry, W. H. Freeman and Co. New York, NY.

162. Sukhija, P. S., and D. L. Palmquist. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem. 36:1202.

163. Sutton, J. D., R. H. Smith, A. B. McAllan, J. E. Storry, and D. A. Corse. 1975. Effect of variations in dietary protein and of supplements of cod-liver oil on energy digestion and microbial synthesis in the rumen of sheep fed hay and concentrates. J. Agric. Sci. (Camb.) 84:317.

164. Sutton, J. D., J. E. Storry, and P. E. Brumby. 1978. Rumen microbial synthesis of long chain fatty acids. Proc. Nutr. Soc. 38:4A.

165. Sutton, J. D., J. E. Storry, and J. W. G. Nicholson. 1970. The digestion of fatty acids in the stomach and intestines of sheep given widely different rations. J. Dairy Res. 37:97.

166. Thompson, G. E. 1989. Mammary extraction of plasma triglycerides in the cow during lactogenesis. Comp. Biochem. Physiol. 94B:411.

167. Tytgat, G. N., C. E. Rubin, and D. R. Saunders. 1971. Synthesis and transport of lipoprotein particles by

intestinal absorptive cells in man. J. Clin. Invest. 50:2065.

168. Uden, P., P. E. Colucci, and P. J. VanSoest. 1980. Investigation of chromium, cerium and cobalt as markers in digesta rate of passage studies. J. Sci. Food Agric. 31:625.

169. Vernon, R. G. 1980. Lipid metabolism in adipose tissue of ruminant animals. Prog. Lipid. Res. 19:23.

170. Vernon, R. G. 1989. Endocrine control of metabolic adaptation during lactation. Proc. Nutr. Soc. 48:23.

171. Viviani, R. 1970. Metabolism of long-chain fatty acids in the rumen. Adv. Lipid Res. 8:267.

172. Waghorn, G. C., and R. L. Baldwin. 1984. Model of metabolite flux within mammary gland of the lactating cow. J. Dairy Sci. 67:531.

173. Waltz, D. M., M. D. Stern, and D. J. Illg. 1989. Effect of ruminal degradation of blood meal and feather meal on the intestinal amino acid supply to lactating cows. J. Dairy Sci. 72:1509.

174. Warner, M. E., J. E. Pratt, and A. M. Novakovic. 1986. Potential Impacts of routing milk to dairy processing plants on the basis of assembly cost and protein content. J. Dairy Sci. 69:1972.

175. White, T. W., R. B. Grainger, F. H. Baker, and J. W. Stroud. 1958. Effect of supplemental fat on digestion and the ruminal calcium requirements of sheep. J. Anim. Sci. 17:797.

176. Wilks, D. L., C. E. Coppock, K. N. Brooks, and C. E. Gates. 1991. Effects of differences in starch content of diets with whole cottonseed or rice bran on milk casein. J. Dairy Sci. 74:1314.

177. Williams, C. H., D. J. Davis, and O. Iismaa. 1962. The determination of chromic oxide in feces by atomic absorption spectrophotometry. J. Agr. Sci. 59:381.

178. Wonsil, B. J. 1990. Biohydrogenation, post ruminal flow and apparent digestibility of dietary lipids in lactating Holstein cows. M. S. Thesis, Virginia Polytechnic Inst. and State Univ., Blacksburg.

179. Yang, Y. T., J. M. Rohde, and R. L. Baldwin. 1978. Dietary lipid metabolism in lactating dairy cows. J. Dairy Sci. 61:1400.

180. Zerbini, E., C. E. Polan, and J. H. Herbein. 1988. Effect of dietary soybean meal and fish meal on protein digesta flow in Holstein cows during early and midlactation. J. Anim. Sci. 52:857.

APPENDICES

Analysis of Variance Tables

Appendix A.

Abbreviations:

*.....P < 0.1

**.....P < 0.05

DF.....degrees of freedom

C vs F.....control vs. fat

Among T.....among treatments

Prot.....protein source

Niac.....niacin

PxN.....protein x niacin

W.....week

P.....protein

N.....niacin

SBM.....soybean meal

UIP.....undegradable intake protein

CTL.....control

Appendix B.

TABLE 14. Analysis of variance for dependent variables in experiment 1.

Daily milk yield, kg/d

SOURCE	DF	MS	F	S				
						SBM	UIP	mean
Treatment	4	95.12	1.62		+N	33.67	31.09	32.38
C vs F	1	98.22	1.67		-N	31.91	32.37	32.14
Among T	3	94.09	1.60		mean	32.79	31.73	
Prot	1	90.86	1.55					
Niac	1	4.43	0.08		CTL	33.50		
PxN	1	186.98	3.19	*				
Error A	35	58.68						
Week	9	155.76	2.96	**				
Error B	63	52.69						
W T	36	4.11	0.07					
W P	9	5.39	0.09					
W N	9	4.82	0.08					
W P N	9	4.35	0.07					
Error C	252	61.44						
TOTAL	399	57.11						

4 % FCM, kg/d

SOURCE	DF	MS	F	S				
						SBM	UIP	mean
Treatment	4	172.07	3.46	*	+N	30.89	27.27	29.08
C vs F	1	144.05	2.90	*	-N	29.42	30.54	29.98
Among T	3	181.41	3.65	**	mean	30.16	28.91	
Prot	1	79.52	1.60					
Niac	1	106.58	2.14		CTL	30.90		
PxN	1	358.11	7.20	**				
Error A	35	49.72						
Week	9	88.93	1.63					
Error B	63	54.72						
W T	36	16.74	0.28					
W P	9	10.48	0.17					
W N	9	18.05	0.30					
W P N	9	13.57	0.22					
Error C	252	60.76						
TOTAL	399	56.62						

Milk fat, %

SOURCE	DF	MS	F	S		SBM	UIP	mean
Treatment	4	2.55	6.06	**	+N	3.36	3.21	3.29
C vs F	1	0.45	1.06		-N	3.45	3.69	3.57
Among T	3	3.26	7.72	**	mean	3.41	3.45	
Prot	1	0.13	0.31					
Niac	1	6.56	15.56	**	CTL	3.51		
PxN	1	3.08	7.30	**				
Error A	35	0.42						
Week	9	0.56	0.94					
Error B	63	0.60						
W T	36	0.66	1.11					
W P	9	0.41	0.68					
W N	9	0.56	0.94					
W P N	9	0.84	1.41					
Error C	252	0.60						
TOTAL	399	0.61						

Milk fat, kg/d

SOURCE	DF	MS	F	S		SBM	UIP	mean
Treatment	4	0.44	4.75	**	+N	1.13	0.99	1.06
C vs F	1	0.29	3.06	*	-N	1.11	1.17	1.14
Among T	3	0.50	5.32	**	mean	1.12	1.08	
Prot	1	0.11	1.23					
Niac	1	0.56	6.04	**	CTL	1.17		
PxN	1	0.81	8.69	**				
Error A	35	0.09						
Week	9	0.11	0.90					
Error B	63	0.12						
W T	36	6.45	51.96	**				
W P	9	0.04	0.29					
W N	9	0.06	0.51					
W P N	9	0.06	0.48					
Error C	252	0.12						
TOTAL	399	0.12						

Milk protein, %

SOURCE	DF	MS	F	S		SBM	UIP	mean
Treatment	4	0.22	3.86	**	+N	3.01	2.95	2.98
C vs F	1	0.38	6.52	**	-N	3.06	3.00	3.03
Among T	3	0.17	2.97	**	mean	3.04	2.98	
Prot	1	0.30	5.19	**				
Niac	1	0.21	3.67	*	CTL	3.08		
PxN	1	0.00	0.07					
Error A	35	0.06						
Week	9	0.22	4.44	**				
Error B	63	0.05						
W T	36	0.02	0.33					
W P	9	0.01	0.27					
W N	9	0.01	0.31					
W P N	9	0.02	0.43					
Error C	252	0.05						
TOTAL	399	0.05						

Milk protein, kg/d

SOURCE	DF	MS	F	S		SBM	UIP	mean
Treatment	4	0.18	3.93	**	+N	1.01	0.91	0.96
C vs F	1	0.27	5.86	**	-N	0.97	0.97	0.97
Among T	3	0.15	3.29	**	mean	0.99	0.94	
Prot	1	0.22	4.75	**				
Niac	1	0.01	0.11		CTL	1.03		
PxN	1	0.23	5.00	**				
Error A	35	0.05						
Week	9	0.08	1.98	*				
Error B	63	0.04						
W T	36	0.00	0.09					
W P	9	0.00	0.11					
W N	9	0.01	0.14					
W P N	9	0.00	0.06					
Error C	252	0.05						
TOTAL	399	0.04						

Milk lactose, %

SOURCE	DF	MS	F	S		SBM	UIP	mean
Treatment	4	0.70	4.54	**	+N	4.83	4.81	4.82
C vs F	1	1.20	7.76	**	-N	4.83	4.66	4.75
Among T	3	0.54	3.46	**	mean	4.83	4.74	
Prot	1	0.76	4.92	**				
Niac	1	0.41	2.66		CTL	4.92		
PxN	1	0.44	2.82					
Error A	35	0.16						
Week	9	0.79	5.15	**				
Error B	63	0.15						
W T	36	0.08	0.46					
W P	9	0.11	0.67					
W N	9	0.06	0.38					
W P N	9	0.09	0.55					
Error C	252	0.17						
TOTAL	399	0.18						

Milk lactose, kg/d

SOURCE	DF	MS	F	S		SBM	UIP	mean
Treatment	4	0.41	2.69	**	+N	1.64	1.49	1.57
C vs F	1	0.80	5.19	**	-N	1.55	1.52	1.54
Among T	3	0.28	1.85		mean	1.60	1.51	
Prot	1	0.55	3.57					
Niac	1	0.01	0.48		CTL	1.66		
PxN	1	0.30	1.94					
Error A	35	0.15						
Week	9	0.73	5.08	**				
Error B	63	0.14						
W T	36	0.02	0.12					
W P	9	0.02	0.13					
W N	9	0.02	0.12					
W P N	9	0.03	0.15					
Error C	252	0.18						
TOTAL	399	0.17						

SNF, %

SOURCE	DF	MS	F	S		SBM	UIP	mean
Treatment	4	1.00	5.42	**	+N	8.52	8.41	8.47
C vs F	1	2.92	15.84	**	-N	8.56	8.46	8.51
Among T	3	0.36	1.94		mean	8.54	8.44	
Prot	1	0.90	4.88	**				
Niac	1	0.17	0.95		CTL	8.70		
PxN	1	0.00	0.11					
Error A	35	0.18						
Week	9	0.54	4.67	**				
Error B	63	0.11						
W T	36	0.04	0.35					
W P	9	0.02	0.15					
W N	9	0.05	0.43					
W P N	9	0.03	0.25					
Error C	252	0.13						
TOTAL	399	0.14						

SNF, kg/d

SOURCE	DF	MS	F	S		SBM	UIP	mean
Treatment	4	1.25	2.99	**	+N	2.87	2.60	2.74
C vs F	1	2.06	4.91	**	-N	2.73	2.74	2.74
Among T	3	0.99	2.35	*	mean	2.80	2.67	
Prot	1	1.33	3.18	*				
Niac	1	0.00	0.00		CTL	2.92		
PxN	1	1.63	3.88	*				
Error A	35	0.42						
Week	9	1.50	4.10	**				
Error B	63	0.37						
W T	36	0.03	0.08					
W P	9	0.04	0.09					
W N	9	0.04	0.08					
W P N	9	0.03	0.08					
Error C	252	0.45						
TOTAL	399	0.43						

Dry matter intake (DMI), kg/d

SOURCE	DF	MS	F	S		SBM	UIP	mean
Treatment	4	16.04	1.88		+N	23.87	23.28	23.58
C vs F	1	37.66	4.41	**	-N	24.05	23.66	23.86
Among T	3	8.83	1.03		mean	23.96	23.47	
Prot	1	19.29	2.26					
Niac	1	6.35	0.74		CTL	22.95		
PxN	1	0.85	0.10					
Error A	35	8.54						
Week	9	3.08	0.37					
Error B	63	8.34						
W T	36	3.87	0.51					
W P	9	6.00	0.79					
W N	9	3.87	0.51					
W P N	9	2.67	0.35					
Error C	252	7.62						
TOTAL	399	7.46						

Mean body weight (BW), kg

SOURCE	DF	MS	F	S		SBM	UIP	mean
Treatment	4	35307	8.74	**	+N	639	621	630
C vs F	1	58287	14.43	**	-N	663	626	645
Among T	3	27647	6.85	**	mean	651	624	
Prot	1	58069	14.38	**				
Niac	1	17201	4.26		CTL	607		
PxN	1	7672	1.90					
Error A	35	4039						
Week	9	1680	0.44					
Error B	63	3811						
W T	36	506	0.12					
W P	9	621	0.15					
W N	9	915	0.22					
W P N	9	341	0.08					
Error C	252	4105						
TOTAL	399	3986						

Plasma glucose, mg/dl

SOURCE	DF	MS	F	S		SBM	UIP	mean
Treatment	4	96.48	1.82		+N	65.66	62.35	64.00
C vs F	1	281.31	5.31	**	-N	64.70	61.12	62.91
Among T	3	34.86	0.66		mean	65.18	61.73	
Prot	1	94.98	1.79					
Niac	1	9.47	0.18		CTL	56.83		
PxN	1	0.14	0.00					
Error	35	52.94						
TOTAL	39							

Plasma ß-hydroxybutyrate, mg/dl

SOURCE	DF	MS	F	S		SBM	UIP	mean
Treatment	4	13.33	2.16		+N	8.74	11.33	10.03
C vs F	1	2.62	0.42		-N	9.58	7.93	8.76
Among T	3	16.89	2.74		mean	9.16	9.63	
Prot	1	1.73	0.28					
Niac	1	13.08	2.12		CTL	8.76		
PxN	1	35.87	5.81	**				
Error	35	6.17						
TOTAL	39							

Plasma cholesterol, mg/dl

SOURCE	DF	MS	F	S		SBM	UIP	mean
Treatment	4	786	0.53		+N	195.1	192.4	193.7
C vs F	1	286	0.19		-N	216.6	199.1	207.9
Among T	3	953	0.64		mean	205.9	195.7	
Prot	1	818	0.55					
Niac	1	1601	1.08		CTL	207.5		
PxN	1	439	0.30					
Error	35	1484						
TOTAL	39							

AD digestibility, %

SOURCE	DF	MS	F	S		SBM	UIP	mean
Treatment	4	143.50	1.89		+N	60.65	65.78	63.21
C vs F	1	184.25	2.43		-N	65.29	70.50	67.90
Among T	3	129.92	1.71		mean	62.97	68.14	
Prot	1	214.09	2.82					
Niac	1	175.64	2.31		CTL	60.19		
PxN	1	0.02	0.00					
Error	35	75.90						
TOTAL	39							

ND digestibility, %

SOURCE	DF	MS	F	S		SBM	UIP	mean
Treatment	4	90.85	2.20	*	+N	72.19	76.46	74.32
C vs F	1	203.69	4.92	**	-N	74.53	78.21	76.37
Among T	3	53.24	1.29		mean	73.36	77.33	
Prot	1	126.29	3.05	*				
Niac	1	33.35	0.81		CTL	69.71		
PxN	1	0.07	0.00					
Error	35	41.39						
TOTAL	39							

CP digestibility, %

SOURCE	DF	MS	F	S		SBM	UIP	mean
Treatment	4	85.65	1.98		+N	74.40	76.65	75.52
C vs F	1	224.77	5.21	**	-N	77.23	79.80	78.51
Among T	3	39.28	0.91		mean	75.82	78.22	
Prot	1	46.22	1.07					
Niac	1	71.40	1.65		CTL	71.09		
PxN	1	0.21	0.00					
Error	35	43.16						
TOTAL	39							

DM digestibility, %

SOURCE	DF	MS	F	S		SBM	UIP	mean
Treatment	4	63.35	1.40		+N	70.82	74.47	72.64
C vs F	1	54.03	1.19		-N	73.86	77.85	75.86
Among T	3	66.46	1.47		mean	72.34	76.16	
Prot	1	116.62	2.57					
Niac	1	82.53	1.82		CTL	71.35		
PxN	1	0.22	0.00					
Error	35	45.31						
TOTAL	39							

Fatty acid digestibility

SOURCE	DF	MS	F	S		SBM	UIP	mean
Treatment	4	141.05	1.93		+N	70.47	76.61	73.54
C vs F	1	231.28	3.16	*	-N	75.80	79.37	77.58
Among T	3	110.97	1.52		mean	73.13	77.99	
Prot	1	188.91	2.58					
Niac	1	130.86	1.79		CTL	81.57		
PxN	1	13.15	0.18					
Error	35	73.19						
TOTAL	39							

Total protein in milk, %

SOURCE	DF	MS	F	S		SBM	UIP	mean
Treatment	4	0.14	0.79		+N	2.82	2.64	2.73
C vs F	1	0.02	0.12		-N	2.81	2.76	2.79
Among T	3	0.18	1.02		mean	2.82	2.70	
Prot	1	0.33	1.89					
Niac	1	0.08	0.48		CTL	2.73		
PxN	1	0.12	0.68					
Error A	35	0.17						
Week	2	0.12	1.33					
Error B	14	0.09						
W T	8	0.04	0.88					
W P	2	0.08	1.90					
W N	2	0.01	0.27					
W P N	2	0.03	0.77					
Error C	56	0.04						
TOTAL	119	0.09						

Casein % in true milk protein

SOURCE	DF	MS	F	S			
					SBM	UIP	mean
Treatment	4	23.29	0.55	+N	85.53	83.31	84.42
C vs F	1	32.33	0.76	-N	84.41	84.14	84.27
Among T	3	20.28	0.48	mean	84.97	83.72	
Prot	1	37.44	0.88				
Niac	1	0.51	0.01	CTL	85.64		
PxN	1	22.88	0.54				
Error A	35	42.31					
Week	2	114.70	3.60	**			
Error B	14	31.89					
W T	8	21.83	1.56				
W P	2	6.32	0.45				
W N	2	20.97	1.50				
W P N	2	54.43	3.88				
Error C	56	14.03					
TOTAL	119	26.97					

Whey % in true milk protein

SOURCE	DF	MS	F	S			
					SBM	UIP	mean
Treatment	4	23.29	0.55	+N	14.47	16.69	15.58
C vs F	1	32.79	0.77	-N	15.59	15.86	15.73
Among T	3	20.12	0.48	mean	15.03	16.28	
Prot	1	37.44	0.88				
Niac	1	0.05	0.00	CTL	14.36		
PxN	1	22.88	0.54				
Error A	35	42.31					
Week	2	114.70	3.60	**			
Error B	14	31.89					
W T	8	21.83	1.56				
W P	2	6.32	0.45				
W N	2	20.97	1.50				
W P N	2	54.43	3.88	*			
Error C	56	14.03					
TOTAL	119	26.97					

NPN % of total milk nitrogen

SOURCE	DF	MS	F	S
Treatment	4	11.50	0.62	
C vs F	1	2.63	0.14	
Among T	3	14.46	0.78	
Prot	1	2.48	0.13	
Niac	1	8.58	0.46	
PxN	1	32.32	1.73	
Error A	35	18.65		
Week	2	69.71	15.66	**
Error B	14	4.45		
W T	8	3.59	0.44	
W P	2	0.70	0.09	
W N	2	5.38	0.66	
W P N	2	5.99	0.73	
Error C	56	8.19		
TOTAL	119	11.66		

	SBM	UIP	mean
+N	18.03	17.19	17.61
-N	16.27	17.75	17.01
mean	17.15	17.47	
CTL	16.94		

Appendix C.

TABLE 15. Analysis of variance for dependent variables in experiment 2.

Crude protein delivered to the duodenum, %

SOURCE	DF	MS	F	S		SBM	UIP	mean
Cow	7	1.86	1.05		+N	11.06	10.07	10.56
Treatment	4	7.05	3.98	**	-N	10.85	10.41	10.63
C vs F	1	23.47	13.26	**	mean	10.96	10.24	
Among T	3	1.58	0.89					
Prot	1	4.10	2.32		CTL	12.51		
Niac	1	0.04	0.02					
PxN	1	0.59	0.34					
Error	28	1.77						
TOTAL	39							

Dry matter digestibility, %

SOURCE	DF	MS	F	S		SBM	UIP	mean
Cow	7	18.40	0.66		+N	64.30	63.70	64.00
Treatment	4	65.60	2.34		-N	66.98	65.74	66.36
C vs F	1	210.42	7.51	**	mean	65.64	64.72	
Among T	3	17.36	0.62					
Prot	1	6.73	0.24		CTL	70.91		
Niac	1	44.52	1.59					
PxN	1	0.83	0.03					
Error	28	28.00						
TOTAL	39							

AD digestibility, %

SOURCE	DF	MS	F	S		SBM	UIP	mean
Cow	7	27.90	0.42		+N	52.45	51.63	52.04
Treatment	4	50.50	0.75		-N	55.54	54.50	55.02
C vs F	1	123.97	1.84		mean	54.00	53.06	
Among T	3	25.98	0.39					
Prot	1	6.94	0.10		CTL	57.93		
Niac	1	70.89	1.05					
PxN	1	0.10	0.00					
Error	28	67.20						
TOTAL	39							

ND digestibility, %

SOURCE	DF	MS	F	S		SBM	UIP	mean
Cow	7	17.60	0.59		+N	68.35	68.14	68.24
Treatment	4	20.40	0.68		-N	70.83	70.14	70.49
C vs F	1	39.18	1.31		mean	69.59	69.14	
Among T	3	14.11	0.47					
Prot	1	1.64	0.05		CTL	71.84		
Niac	1	40.24	1.35					
PxN	1	0.45	0.02					
Error	28	29.90						
TOTAL	39							

Fatty acid digestibility, %

SOURCE	DF	MS	F	S		SBM	UIP	mean
Cow	7	176.8	1.94		+N	55.99	54.28	55.13
Treatment	4	649.0	7.12	**	-N	60.69	56.85	58.77
C vs F	1	2419.5	26.53	**	mean	58.34	55.56	
Among T	3	58.9	0.65					
Prot	1	61.8	0.68		CTL	76.39		
Niac	1	105.8	1.16					
PxN	1	9.1	0.10					
Error	28	91.2						
TOTAL	39							

CP digestibility, %

SOURCE	DF	MS	F	S		SBM	UIP	mean
Cow	7	9.70	0.49		+N	72.37	74.30	73.34
Treatment	4	12.00	0.60		-N	74.95	74.82	74.88
C vs F	1	14.05	0.70		mean	73.66	74.56	
Among T	3	11.35	0.57					
Prot	1	6.41	0.32		CTL	72.63		
Niac	1	19.16	0.96					
PxN	1	8.49	0.42					
Error	28	20.00						
TOTAL	39							

Niacin in rumen fluid, ug/ml

SOURCE	DF	MS	F	S		SBM	UIP	mean
Cow	7	247	0.95		+N	23.41	18.45	20.93
Treatment	4	2030	7.85	**	-N	8.80	9.12	8.96
C vs F	1	1885	7.29	**	mean	16.11	13.79	
Among T	3	2078	8.04	**				
Prot	1	215	0.83		CTL	7.71		
Niac	1	5739	22.20	**				
PxN	1	280	1.08					
Error A	28	259						
Hour	4	2279	19.72	**				
Error B	28	116						
Trt*Hour	16	724	5.23	**				
H P	4	31	0.22					
H N	4	2145	15.50	**				
H N P	4	161	1.16					
Error C	112	138						
TOTAL	199							

Rumen fluid pH

SOURCE	DF	MS	F	S		SBM	UIP	mean
Cow	7	0.68	1.67		+N	6.30	6.28	6.29
Treatment	4	0.18	0.45		-N	6.26	6.31	6.29
C vs F	1	0.62	1.52		mean	6.28	6.30	
Among T	3	0.04	0.09					
Prot	1	0.03	0.06		CTL	6.17		
Niac	1	0.03	0.07					
PxN	1	0.06	0.15					
Error A	28	0.41						
Hour	6	1.65	47.16	**				
Error B	42	0.04						
Trt*Hour	24	0.04	1.06					
H P	6	0.01	0.33					
H N	6	0.09	2.70	**				
H N P	6	0.01	0.37					
Error C	168	0.03						
TOTAL	199							

Rumen fluid ammonia nitrogen (NH_3-N), ug/ml

SOURCE	DF	MS	F	S		SBM	UIP	mean
Cow	7	63416	3.71	**	+N	214.7	231.0	222.9
Treatment	4	22598	1.32		-N	220.5	188.7	204.6
C vs F	1	35868	2.10		mean	217.6	209.9	
Among T	3	18174	1.06					
Prot	1	3336	0.20		CTL	185.4		
Niac	1	18741	1.10					
PxN	1	32446	1.90					
Error A	28	17082						
Hour	6	112718	38.37	**				
Error B	42	2938						
Trt*Hour	24	1210	0.61					
H P	6	862	0.43					
H N	6	2984	1.49					
H N P	6	725	0.36					
Error C	168	2000						
TOTAL	199							

Duodenal fluid ammonia nitrogen (NH_3-N), ug/ml

SOURCE	DF	MS	F	S		SBM	UIP	mean
Cow	7	8894	0.70		+N	126.4	95.0	110.7
Treatment	4	14721	1.16		-N	109.1	88.2	98.7
C vs F	1	10884	0.86		mean	117.7	91.6	
Among T	3	16000	1.26					
Prot	1	38333	3.03	*	CTL	89.09		
Niac	1	8108	0.64					
PxN	1	1559	0.12					
Error A	28	12660						
Hour	6	4840	14.67	**				
Error B	42	330						
Trt*Hour	24	227	0.75					
H P	6	190	0.63					
H N	6	292	0.96					
H N P	6	260	0.86					
Error C	168	303						
TOTAL	199							

Total VFA in rumen fluid, umol/ml

SOURCE	DF	MS	F	S		SBM	UIP	mean
Cow	7	6143	2.24	*	+N	133.6	123.6	128.6
Treatment	4	3682	1.34		-N	112.5	116.0	114.3
C vs F	1	66	0.02		mean	123.1	119.8	
Among T	3	4887	1.78					
Prot	1	596	0.22		CTL	122.7		
Niac	1	11500	4.19	*				
PxN	1	2564	0.93					
Error A	28	2746						
Hour	6	13310	25.48	**				
Error B	42	522						
Trt*Hour	24	294	0.74					
H P	6	107	0.27					
H N	6	330	0.83					
H N P	6	218	0.55					
Error C	168	398						
TOTAL	199							

A:P ratio

SOURCE	DF	MS	F	S		SBM	UIP	mean
Cow	7	6.54	2.07	*	+N	4.21	3.71	3.96
Treatment	4	5.34	1.69		-N	3.80	3.45	3.63
C vs F	1	4.22	1.33		mean	4.01	3.58	
Among T	3	5.71	1.81					
Prot	1	10.25	3.24	*	CTL	4.10		
Niac	1	6.57	2.08					
PxN	1	0.32	0.10					
Error A	28	3.16						
Hour	6	6.91	84.02	**				
Error B	42	0.08						
Trt*Hour	24	0.08	0.93					
H P	6	0.10	1.06					
H N	6	0.12	1.28					
H N P	6	0.02	0.18					
Error C	168	0.09						
TOTAL	199							

Acetate, umol/ml

SOURCE	DF	MS	F	S		SBM	UIP	mean
Cow	7	195.71	5.62	**	+N	66.21	64.64	65.43
Treatment	4	49.34	1.42		-N	64.61	63.88	64.25
C vs F	1	35.48	1.02		mean	65.41	64.26	
Among T	3	53.95	1.55					
Prot	1	73.70	2.12		CTL	65.73		
Niac	1	78.06	2.24					
PxN	1	10.10	0.29					
Error A	28	34.80						
Hour	6	67.06	48.66	**				
Error B	42	1.38						
Trt*Hour	24	2.15	1.44					
H P	6	1.14	0.76					
H N	6	3.14	2.10					
H N P	6	0.87	0.58					
Error C	168	1.50						
TOTAL	199							

Propionate, umol/ml

SOURCE	DF	MS	F	S		SBM	UIP	mean
Cow	7	111.02	2.11	*	+N	16.39	18.10	17.25
Treatment	4	93.10	1.77		-N	17.60	19.53	18.57
C vs F	1	88.54	1.69		mean	17.00	18.82	
Among T	3	94.62	1.80					
Prot	1	185.51	3.53	*	CTL	16.50		
Niac	1	97.69	1.86					
PxN	1	0.66	0.01					
Error A	28	52.50						
Hour	6	77.63	66.58	**				
Error B	42	1.17						
Trt*Hour	24	0.65	0.73					
H P	6	0.34	0.38					
H N	6	1.16	1.29					
H N P	6	0.35	0.39					
Error C	168	0.90						
TOTAL	199							

Isobutyrate, umol/ml

SOURCE	DF	MS	F	S
Cow	7	0.39	1.20	
Treatment	4	0.37	1.14	
C vs F	1	0.00	0.00	
Among T	3	0.49	1.52	
Prot	1	0.02	0.06	
Niac	1	0.05	0.15	
PxN	1	1.41	4.36	**
Error A	28	0.32		
Hour	6	0.99	39.20	**
Error B	42	0.03		
Trt*Hour	24	0.02	0.94	
H P	6	0.02	1.01	
H N	6	0.01	0.35	
H N P	6	0.04	2.19	*
Error C	168	0.02		
TOTAL	199			

	SBM	UIP	mean
+N	1.06	1.20	1.13
-N	1.19	1.01	1.10
mean	1.13	1.11	
CTL	1.11		

Butyrate, umol,ml

SOURCE	DF	MS	F	S
Cow	7	15.28	0.92	
Treatment	4	14.11	0.85	
C vs F	1	16.58	1.00	
Among T	3	13.29	0.80	
Prot	1	32.88	1.99	
Niac	1	4.21	0.25	
PxN	1	2.78	0.17	
Error A	28	16.56		
Hour	6	1.20	2.39	
Error B	42	0.50		
Trt*Hour	24	0.65	1.17	
H P	6	0.70	1.26	
H N	6	0.62	1.12	
H N P	6	0.21	0.37	
Error C	168	0.56		
TOTAL	199			

	SBM	UIP	mean
+N	13.12	12.58	12.85
-N	13.07	12.08	12.58
mean	13.10	12.33	
CTL	13.32		

Isovalerate, umol/ml

SOURCE	DF	MS	F	S		SBM	UIP	mean
Cow	7	3.31	3.04	**	+N	1.63	1.75	1.69
Treatment	4	0.57	0.52		-N	1.80	1.55	1.68
C vs F	1	0.03	0.03		mean	1.72	1.65	
Among T	3	0.74	0.68					
Prot	1	0.26	0.24		CTL	1.71		
Niac	1	0.02	0.02					
PxN	1	1.96	1.79					
Error A	28	1.09						
Hour	6	2.01	36.25	**				
Error B	42	0.06						
Trt*Hour	24	0.03	0.95					
H P	6	0.03	0.74					
H N	6	0.01	0.21					
H N P	6	0.06	1.74					
Error C	168	0.04						
TOTAL	199							

Valerate, umol/ml

SOURCE	DF	MS	F	S		SBM	UIP	mean
Cow	7	4.54	4.10	**	+N	1.58	1.72	1.65
Treatment	4	1.17	1.05		-N	1.73	1.94	1.84
C vs F	1	0.94	0.85		mean	1.66	1.83	
Among T	3	1.24	1.12					
Prot	1	1.81	1.64		CTL	1.30		
Niac	1	1.85	1.67					
PxN	1	0.06	0.06					
Error A	28	1.11						
Hour	6	1.41	43.12	**				
Error B	42	0.03						
Trt*Hour	24	0.03	0.84					
H P	6	0.01	0.25					
H N	6	0.03	0.95					
H N P	6	0.04	1.28					
Error C	168	0.03						
TOTAL	199							

Total bacteria, log/ml

SOURCE	DF	MS	F	S		SBM	UIP	mean
Cow	7	0.01	1.45		+N	10.84	10.98	10.91
Treatment	4	0.06	10.39	**	-N	10.89	11.10	11.00
C vs F	1	0.06	10.12	**	mean	10.87	11.04	
Among T	3	0.06	10.48	**				
Prot	1	0.15	26.73	**	CTL	10.82		
Niac	1	0.02	3.72	*				
PxN	1	0.01	1.00					
Error	12	0.01						
TOTAL	23							

Cellulolytic bacteria, log/ml

SOURCE	DF	MS	F	S		SBM	UIP	mean
Cow	7	2.57	1.31		+N	6.80	7.31	7.06
Treatment	4	4.91	2.52	*	-N	7.20	6.84	7.02
C vs F	1	17.72	9.08	**	mean	7.00	7.08	
Among T	3	0.64	0.33					
Prot	1	0.09	0.05		CTL	8.73		
Niac	1	0.04	0.02					
PxN	1	1.78	0.91					
Error	28	1.95						
TOTAL	39							

Protozoa, log/ml

SOURCE	DF	MS	F	S		SBM	UIP	mean
Cow	7	0.11	1.10		+N	5.39	5.46	5.42
Treatment	4	0.03	0.25		-N	5.54	5.47	5.51
C vs F	1	0.01	0.08		mean	5.47	5.47	
Among T	3	0.03	0.31					
Prot	1	0.00	0.00		CTL	5.50		
Niac	1	0.05	0.52					
PxN	1	0.04	0.40					
Error	28	0.10						
TOTAL	39							

Liquid dilution rate, slope comparison

SOURCE	DF	MS	S		SBM	UIP	mean
Cow	7	2E-04	1.78	+N	0.070	0.069	0.070
Treatment	4	5E-05	0.50	-N	0.066	0.075	0.070
C vs F	1	1E-04	1.18	mean	0.068	0.072	
Among T	3	3E-05	0.28				
Prot	1	2E-05	0.23	CTL	0.066		
Niac	1	3E-05	0.25				
PxN	1	4E-05	0.35				
Error	28	1E-04					
TOTAL	39						

Dry matter rate of passage, slope comparison

SOURCE	DF	MS	S		SBM	UIP	mean
Cow	7	1E-04	1.22	+N	0.015	0.024	0.019
Treatment	4	1E-04	0.98	-N	0.020	0.020	0.020
C vs F	1	2E-05	0.19	mean	0.017	0.022	
Among T	3	1E-04	1.24				
Prot	1	2E-04	2.05	CTL	0.018		
Niac	1	3E-06	0.03				
PxN	1	2E-04	1.65				
Error	28	1E-04					
TOTAL	39						